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PARVOBACTERIA

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This group includes heterogeneous small gram negative fastidious coccobacilli .

They are non motile non spore forming aerobic and microaerophilic bacteria.

It includes Haemophilus , Bordetella and Brucella.

HAEMOPHILUS

This genus includes small gram negative coccobacilli that require enriched media usually containing blood or its derivatives for its isolation . These factors are known as X factor which is hemin factor .

V factor which is Niacin Adenine Dinucleotide NAD .

Some of them resembles flora of conjunctiva and mucous membranes while others are pathogenic.

- Pathogenic species are :

- *Haemophilus influenza*
- *Haemophilus ducreyi*
- *Haemophilus aegypticus*

Haemophilus influenza

Organisms within this genus are Gram negative coccobacilli ,it requires both X and V factors for their growth .

Their mean size is about 1.5 x 0.3 ㎛.

- Non pathogenic strain of this species is non capsulated and it is known as *Haemophilus influenza* Type A , it is found on the mucous membrane of the upper respiratory tract in humans .

- While the pathogenic strain is known as *Haemophilus influenza* type B and its capsulated and it is considered as an important cause of meningitis in children and occasionally cause respiratory tract infection in children and adults . Also it cause conjunctivitis , septicemia , subacute endocarditis and septic arthritis .

Growth Characters

- It is fastidious organism and needs specific requirements for growth like X and V factors. It grows much larger around *Staphylococcus aureus* colonies and shows what is known as Satellitism phenomenon due to V factor synthesis by Staphylococcus.

Variation

- *Haemophilus influenza* has marked tendency to lose its capsule . Transformation is recognized in this organism.
- Plasmid mediated antibiotic resistance is seen for Ampicillin and Chloramphenicol.

Antigenic Structure

1- Capsular Antigen:

- There are two types of *Haemophilus influenza :*
- *Type A which is non capsulated*
- *Type B which is capsulated and the capsular antigen is* ***Poly saccharide*** *(Polyribose Ribitol Phosphate PRP) ,Regarding this antigen there are 6 types known as* ***A, b , c ,d, E& F.***

2-Somatic Ag.

- Somatic Ag.is protein in nature and the endotoxin which is lipopolysaccharide in nature .
- Type B is usually causes 95% of disease while other types are rarely causing disease.

Pathogenicity and Clinical Findings

- Normally the non capsulated organisms inhabit human respiratory tract and nasopharynx . Capsulated type produces primary infection and may cause sinusitis , laryngeo - tracheitis ,epiglottitis ,otitis and in young children it causes meningitis.

- The organisms reach blood in case of untreated patients leading to septicemia, the more complicated case is meningitis and it may establish joints causing septic arthritis.
- *Haemophilus influenza* meningitis occurs in children between 5 months – 5 years .before 5 months baby has maternal immunity and above 5 years bacterial antibody will develop so the disease is rare in adults.

Lab diagnosis

- Clinical specimens depends on the site of infection and the type of the clinical symptoms.
- Nasopharyngeal swab , sputum ,blood ,CSF and pus in case of sinusitis and otitis. Each specimen undergo investigation by :

DIRECT INVESTIGATION USING:

A- Gram stained smear , positive findings show gram negative coccobacilli

B-Fluorescent antibody test for smears

C-Capsule swelling test using capsular antibody in the same way of that of *Streptococcus pneumoniae* capsular quelling reaction .

Indirect investigation

- Indirect investigation testing through cultivation of the specimen on chocolate agar or other media enriched with X and V factors for 24 hours at 37c . Colonies are small glistening like due drops.
- To confirm Molecular diagnostic methods can be used like PCR.

Haemophilus aegypticus

- It Is formerly called Koch's week bacillus and it has been associated with highly communicable form of conjunctivitis (pink eye) . It can be recover from pneumonic patients .

Haemophilus ducreyi

- It causes soft chancre , a sexually sex transmitted disease STD .The chancroid lesion consists of ulcer on the external genitalia with marked swelling of regional lymph nodes and
- It must be differentiated from syphilis. It requires only X factor for growth. Treatment with sulfonamide or oral erythromycin often results in healing within 2 weeks.

Haemophilus parainfluenza

- It is found normally in the respiratory tract of human and it resembles *Haemophilus influenza* in characters. It requires V factor for growth. It has been encountered in disease rarely and mainly infective endocarditis.

Haemophilus aphrophilus

- It is normally found in the oral and respiratory tract ,infections are frequent, endocarditis , brain abscess and meningitis.
- X factor only is required for its growth and CO2 5% enhance its growth.

Bordetella

- There are three species of Bordetella :

- *Bordetella pertusis*
- *Bordetella parapertusis*
- *Bordetella bronchoseptica*

Bordetella pertusis

- It is gram negative coccobacillus resembling *Haemophilus influenza* but do not require X and V factors for its growth.
- Bipolar staining technique with toluidine blue dye and metachromatic granules can be detected. Capsule is present .

Cultural characters

Primary isolation requires enriched medium known as Bordet –Gengou Medium , it contains glycerol, potato extract , glycerol and agar, in addition to that 0.5 ᵐg/ml of penicillin. A charcoal containing medium Similar to that used for the isolation of *Legionella pneumophilia*. Suitable incubation period is 3-7 days at 35-37C in moist environment

- Mercury drop colonies are produced on Bordet – Gengou Medium.
- A narrow zone of beta hemolysis on blood agar is associated with virulent *Bordetella pertusis*.

VARIATION

- The organism on first isolation from patients on enriched medium shows smooth colonies capsulated organisms and this phase is known as phase -1
- Phase-2 and phase-3 are intermediate .
- Phase-4 is characterized by rough colonies and non capsulated and non toxigenic and non pathogenic strain.

Antigenic structure:

1-Pertusis toxin : It is the major virulence factor and elicits immunity.

2-Histamine sensitizing Antigen : It is responsible for paroxysmal cough .

3-Haemagglutinins : They are fimbrial Haemagglutinins , leucocytosis promoting factor promotes lymphocytosis .

Phase-1 usually contains large amount of protective antigen than other phases.

Pathogenesis :

- *Bordetella pertusis* is an obligate human pathogen causing whooping cough which is an acute child respiratory disease.
- The organisms survive for short period outside the body , transmission is mainly by respiratory route from early cases and possibly through carriers . The organism adheres to the epithelial cells of trachea and bronchi and multiply rapidly on the site of adhesion . It interferes with ciliary action .

- Bacteria liberate toxins and substances that irritate surface cells , later necrosis and lymphocytosis with peri-bronchial inflammation and interstitial pneumonia . Secondary invaders like *Staphylococcus aureus* and *H. influenza* may give rise to bacterial pneumonia . obstruction of smaller bronchioles by mucous plugs results in atelectasis and diminished oxygenation of the blood and this contributes convulsion frequency.

Clinical findings :

- After an incubation period of about 2 weeks , pertusis occurs in three stages :

A- Catarrhal stage :

- It is characterized by mild cough and sneezing , the patient is highly infectious in this phase due to high number of bacteria in the droplets .

B- Paroxysmal stage :

- Cough increases in severity and show its explosive characters and whoops in inhalation. It may be associated with vomiting , cyanosis and convulsion. Whoop is prominent in infants while paroxysm dominates in older children and adults.

C- Convalescent stage :

- Frequency and severity of cough starts decrease gradually . Several types of adenoviruses and Chlamydia trachomatis can produce clinical picture of *Bordetella pertusis*.

Diagnosis :

- Specimens like nasopharyngeal swab or cough droplets can be cultivated on to Brdet-Gengou agar or blood- charcoal agar.
- Direct examination of the specimen using fluorescent antibody technique (FAT).
- Serology also like ELISA can be used to diagnose pertusis.

Treatment &Prophylaxis :

- Erythromycin , Ampicillin and Tetracycline are effective against this infection .
- Prophylaxis : Killed (smooth phase-1)vaccine is quite protective, every baby should receive injection of killed phase -1 vaccine combined with tetanus and diphtheria a triple vaccine .

Bordetella parapertusis :

- It is similar to whooping cough , it is isolated from patients with acute respiratory infections. Colonies are larger, it is sharing somatic antigen with *Bordetella pertusis* so that they give cross agglutination reaction with *Bordetella pertusis*.

Bordetella bronchoseptica

- It is motile and it may be isolated on blood agar and chocolate agar . It causes mild disease similar to that of *Bordetella parapertusis* .

Brucella

- These are obligate pathogens for animals and humans, they are intracellular pathogens and are causing undulant fever or brucellosis . The most important species associated with zoonotic infections is *Brucella melitensis* typically infects goats and first isolated from spleen of patient in Malta who was dying from fever.

- Brucella abortus , it infects cattles primarily and causes contagious abortion for them.
- Brucella suis , it infects swine
- Brucella canis ,it infects dogs. Brucellosis is primarily an animal disease occasionally infects humans as zoonotic disease through consumption of unpasteurized milk products and meat or contact with infected animals. People at high risk are farmers , workers in slaughter houses and veterinarians.

Morphology and cultural characters :

- These organisms are Gram negative coccobacilli non motile , non spore forming aerobic except certain species needs CO2 for there growth like *Br.abortus* .
- capsule can be demonstrated in smooth and mucoid variants. Brucella needs complex nutritional requirements , they grow on Trypticase Soy agar , blood agar and Castaneda medium.

- They are catalase , oxidase positive while H_2S is released by some strains . Some variants grow in the presence of basic dyes .
- Colonies are small convex appear on enriched media within 2-5 days. Brucellae are moderately sensitive to heat and acidity . they are killed in milk by pasteurization.

Antigenic structure :

- There are two antigens A &M , *Brucella abortus* contains antigen A more than antigen M while *Brucella melitensis* contains more antigen M. In addition to these antigens , a superficial L antigen has been detected resembles Vi antigen of Salmonella.
- Species differentiation among Brucella is made by their sensitivity to dyes and H2S production.

Pathogenesis and pathology :

- Although each species of Brucella has a preferred host, all can infect a wide range of animals , including humans .
- The main routes of infection are :
- 1- Oral route through ingestion of infected milk and milk products and its most common in humans. Cheese made from unpasteurized milk is common source.

- 2- Mucous membranes of respiratory tract through inhalation of contaminated droplets as in risky group infections.
- 3-Skin abrasions through contact with infected animal tissue as an occupational risk factor .
- 4-Man to man contact is very rare .

- The organisms progress from the portal of entry , via lymphatic channels and regional lymph nodes to the thoracic duct and the blood stream ,which distributes them to the paranchyamtous tissue. Granulomatous nodules may develop into abscess form in lymphatic tissue , liver , spleen , bone marrow and other parts of reticuloendothelial system .

- Organisms are seen intracellular. Osteomyelitis meningitis or cholecystitis also occasionally occur The main histologic reaction in brucellosis consists of proliferation of mononuclear cells , fibrin exudates , necrosis and fibrosis . the granulomas consists of epitheoid and giant cells with central necrosis and peripheral fibrosis .

Pathogenicity of Brucella melitensis is more severe than other species. B. abortus usually causes mild disease without suppurative complications and non caseating granulomas of reticuloendothelial system . placentas and fetal membranes of cattle , swine , sheep and goats contain erythritol , a growth factor for Brucellae.

- The proliferation of organisms in pregnant animals leads to placentitis and abortion in these species .
- There is no erythritol in human placentas and abortion is not part of Brucella infections of humans.

Clinical findings :

The incubation period is 1-6 weeks .

The acute onset is characterized by fever , malaise , weakness , aches, and sweats, the fever is undulant . There may be gastrointestinal and nervous symptoms. Spleenomegaly , hepatomegaly and enlarged lymph nodes . Deep pain and disturbance of motion , particularly in vertebral bodies.

- The chronic stage may develop and characterized by weakness ,aches and low grade fever with psychoneurotic symptoms. Brucellae cannot be isolated from the patient at this time but aggluntition titers may be high .

- **Acute brucellosis :**

Site of entry → Lymphatic → Blood↓ → Undulant fever

(Incubation period is1-6 weeks) ↓

Reticuloendothelial system

(Spleen ,Liver , Bone Marrow)

Clinical or Subclinical ↓

↓

Chronic : Chronisty

Weakness , Fatigue , sweating , joint pain

(Culture is negative but agglutination titers will be high)

Diagram showing Brucellosis course of the disease

Lab diagnosis :

- A-Specimens:
- Blood for culture should be taken during febrile stage of illness .
- Biopsy material for culture could be taken from bone marrow and lymph nodes .
- Serum is used for serological tests.

B-Culture :

soy medium with or without 5% sheep blood ,brain heart infusion medium and chocolate agar. blood culture media. All cultures should be incubated in 5-10% CO2 at 35-37 C and should be observed for 3 weeks before being discarded as negative , liquid Brucella agar was designed to culture Brucellae species. The medium is highly enriched .

- Brucella species grow on commonly used Trypticase media should be blindly subcultured during this time on Trypticase Soy agar . Bone marrow and blood are the specimens from which Brucellae are most often isolated. Media used in semiautomated and automated blood culture systems readily grow Brucellae. isolated organisms are typed by H2S , dye inhibition using (Basic Fuchsin 1/50000).

C-SEROLOGY :

- Serum for serological tests is used . IgM appears early in the disease while IgG appears later.
- A- Agglutination test :
- Rose- Bengal test : It must be done with standardized antigen . IgG titers above 1: 80 indicates active infection .

- B- Mercaptoethanol test :

  The addition of 2Me destroy IgM and leaves IgG for agglutination reaction. So it is useful in detection of chronic infections.

- Blocking Antibodies : These are IgA antibody that interferes with agglutination by IgM and IgG and cause negative serologic reactions in low serum dilutions (Prozone phenomenon) in spite of positive infection .

ELISA test

- IgG, IgM , IgA antibodies may be detected using ELISA assay ,which use cytoplasmic proteins as antigens. This test tends to be more sensitive and specific than agglutination test.

3-skin test :

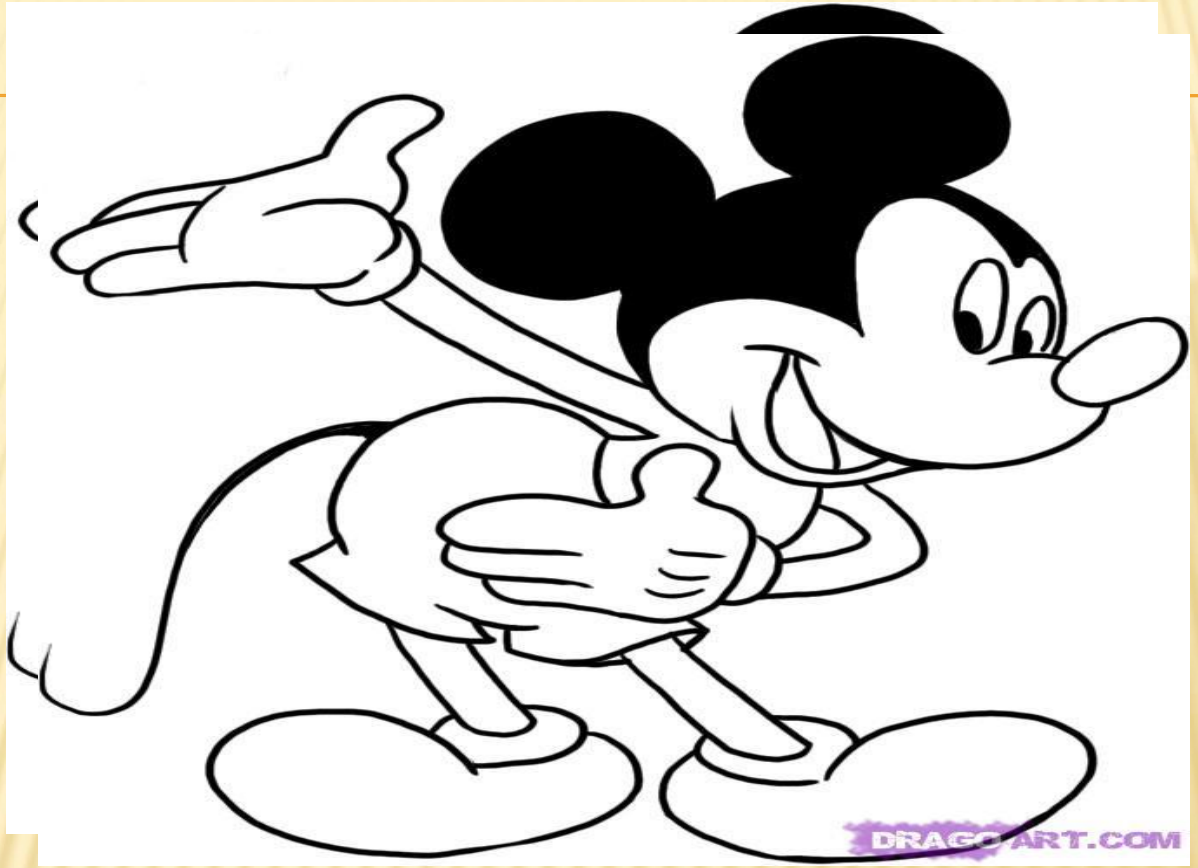
Brucellogen is a protein extract When injected intradermally , edema , erythema and induration develop within 24 hours in case of positive Brucella infection .

Treatment :

- There are different lines for Brucella treatment , different types of antimicrobial agents are dependent her like :
- Tetracycline, doxycycline , Streptomycin , Refadin and Sulfonamide.

Control :

Vaccination of humans and animals in endemic areas.



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