

YERSINIA-BRUCELLA

Learning objectives:

By the end of this lecture , the student should be able to :-

- 1-Define genus Yersinia.
- 2-Discuss mode of transmission and types of plague.
- 3-Describe cultural characters and antigenic structure.
- 4-Outline laboratory diagnosis of different plague types.
- 5-Outline treatment and prevention measures
- 6- Define genus Brucella and enumerate important species.
- 7- Discuss mode of transmission.
- 8- State morphology, cultural characters and antigenic structure.
- 9- Outline laboratory diagnosis and treatment.

Genus Yersinia are short, pleomorphic, gram-negative bacilli, 1-2 μ long, showing bipolar staining, some are capsulated. They are facultative anaerobes, non sporing, may produce acid in carbohydrates, catalase positive and oxidase negative.

1. Yersinia pestis

Causes plague in man and rodents, particularly rats. It is found all over the world as a parasite of rats and other rodents. The organism is transmitted from rat to rat by fleas.

Plague in Man:

If human beings are in close proximity to infected rats, they may be bitten by an infected flea and the organisms are inoculated into the skin. The inoculated organisms may be phagocytosed by polymorphnuclear cells and monocytes where they multiply by producing antiphagocytic proteins and thus resisting phagocytosis. The organisms reach the lymph node through the lymphatics causing a haemorrhagic inflammation

The incubation period is from 2-10 days. The onset is sudden with fever, chills and prostration. There are three forms of plague:

1.Bubonic plague: The lymphatic glands in the groin and less commonly in the axilla or neck swell (buboes) and become very painful. The mortality rate is about 50–70%

2.Pneumonic plague: Symptoms are very severe. The lungs are consolidated and the sputum contains large number of the organisms. Infection usually occurs by inhalation of droplets from patients. The mortality rate is 100%.

3.Septicaemic plague: With a very high level of bacteraemia early in disease before the buboes evolve. Symptoms are very severe. The organisms are present in large number in the blood. Mortality rate is 100%

Morphology:

Gram negative, plump-shaped coccobacilli, it is capsulated when the organism grows at 37 °C or when present in tissues.

When the organism is stained with a weak stain (Methylene blue) the bipolar staining character of the organism appears. It is non-motile and non-sporing.

Culture:

Aerobes and facultative anaerobe. Optimum temperature for growth on primary culture is 27 °C. On agar, the colonies are very small, transparent, white circular discs, later they grow to a varying degree giving the impression of a mixed growth. The organism can grow in the presence of bile (MacConkey's medium). Growth is more rapid in media containing blood or tissue fluids.

Antigenic Structure:

1. Lipopolysaccharide (LPS) endotoxin.
2. Virulence factors: many antigens and toxins
 - a) Envelope antigen: protein-fraction 1, produced at 37 °C. It is antiphagocytic, and activates complement.
 - b) V-W antigens: encoded by 72 Kilobase plasmid -Present in virulent wild strains.
 - c) Coagulase enzyme, acting at 28 °C (flea temperature) but not at 35°C.

Laboratory Diagnosis:

A) Bubonic plague

Fluid or bubo juice is aspirated from the bubo with a syringe. This fluid is examined microscopically, cultured and inoculated into animals:

1. Microscopical examination: Films are stained by the gram's method and methylene blue. A large number of ovoid bipolar stained bacilli are seen. Also examined with specific immunofluorescent stains.
2. Cultures are made on blood agar, MacConkey's agar and the colonies investigated biochemically and by immunofluorescence.
3. Animal inoculation: Some of the bubo juice is injected subcutaneously into rats, mice or guinea pigs. The animal dies within few days. Post-mortem examination reveals local inflammation with necrosis and oedema. Bacilli are present in large number in the blood (septicaemia), local lesion, local bubo and in the spleen.
4. Serology : In unvaccinated patients, an antibody titre of 16 or more is presumptive evidence of *Yersinia pestis* infection. A rising titer confirms the serologic diagnosis.

B) Pneumonic plague:

The patient's sputum is examined microscopically, cultured and inoculated into animals. The sputum is better applied to the nasal mucosa or to a shaved area of skin. This is to avoid infection with other virulent organisms which may be present in the sputum as pneumococci. In addition, serology provides a presumptive evidence for diagnosis.

C) Septicaemic plague

The bacillus may be isolated by the blood culture.

Prevention:

-Vaccination:

1. Haffkine's vaccine: Heat killed suspension of *Yersinia pestis*. Two doses: 1ml and 2 ml are injected subcutaneously with an interval of 7- 10 days. The vaccine gives some protection.
2. The vaccine used now is made from a capsulated strain, killed by formaldehyde, in two doses.
3. Living vaccines made from attenuated or avirulent strains have been tried in animals.

- Chemoprophylaxis may be achieved with tetracycline.

Treatment:

Streptomycin is the drug of choice and is given in very large doses in the first 48 hours.

Tetracycline may be used. Treatment must be continued for at least ten days to avoid relapse. Sometimes both are combined.

BRUCELLA

Brucella are gram-negative bacilli which are parasites of animals and man . They are located intracellularly.

Man becomes infected by contact with animals or their products and the disease is known as **Brucellosis, undulant fever** or **Malta fever**.

There are three species:

- (a) **Brucella melitensis** which affects mainly goats (Malta fever) , sheep and sometimes cows.
- (b) **Brucella abortus** which infects cows.
- (c) **Brucella suis** which infects pigs and includes two types of strains: the Danish type - American type.

In goats and cows: The organism causes bacteraemia, sometimes abortion if the animal is pregnant (contagious abortion). The bacilli pass to the mammary glands where they remain and are excreted in the milk for long time.

Man: Becomes infected by drinking unpasteurized milk or eating fresh butter or cheese from infected milk; also by contact with infected vaginal discharge and urine of animals or with infected carcasses. Infection occurs mainly in farmers, veterinary doctors and butchers. It may also occur through inhalation.

Methods of Infection in Man:

It occurs mainly through the mucous membrane of the alimentary tract; it may occur through abrasions in the skin. Laboratory infections are very common. The organisms pass through the lymphatics, reach the blood and localize in the reticulo-endothelial system where they are able to grow intracellularly and produce granulomatous nodules which may later form abscesses in the liver, spleen and bone marrow.

The incubation period is from 1-6 weeks. The disease is characterized by an acute bacteraemic phase followed by a chronic phase that may last many years. The onset is insidious, the fever may be undulating hence the name, or intermittent or continued.

There is malaise, weakness, aches, profuse sweating and constipation. Gastrointestinal and nervous symptoms may occur, also deep pains and disturbance of motion.

In the chronic stage weakness, pain, low fever and nervousness may be present.

Morphology:

Brucella are small gram negative round or oval coccobacilli, and also in the form of short bacilli. They are non-motile.

Culture:

Strict aerobe but *B. abortus* needs 5 – 10 % CO₂. Optimum temperature for growth is 38 °C. The organism grows on liver extract agar, serum agar and blood agar. Small convex, smooth colonies appear after 2 – 5 days.

Biochemical Reaction:

They are relatively inactive biochemically neither acid nor gas is produced in the sugars.

Resistance:

They are killed by 60 °C in ten minutes and thus they are destroyed by pasteurization of the milk. In fresh cheese undergoing lactic acid fermentation the organisms are killed in a few days. They remain alive in infected butter for several days. They are sensitive to sulpha, streptomycin, tetracycline, and chloramphenicol.

Antigenic Characters:

The three species are very closely related antigenically. An *abortus* antiserum will agglutinate a *Brucella melitensis* suspension and a *Brucella melitensis* antiserum will agglutinate a *Brucella abortus* suspension.

Laboratory Diagnosis:

A) Blood culture

Should be done repeatedly in suspected cases. It is positive 30-50% of cases. Three sulphonated broth tubes are inoculated with the patient's blood.

1. A blood broth tube incubated aerobically.
2. A blood broth tube incubated in air plus 10% CO₂
3. A blood broth tube incubated anaerobically.

Subcultures are made every few days on blood agar plates which are incubated for at least two days and the resulting colonies investigated according to the systematic lines of study. The blood culture tubes are retained for three weeks in the incubator before giving a negative result.

B) Tube agglutination test (usually included in Widal test)

The patient's serum may give a positive reaction after 7-10 days from the beginning of the disease. In the acute stage of the disease agglutinating antibodies may give high titers (1,000 or over). A titer of 80 or more is suggestive. Paired serum samples taken 2 weeks apart may show a rising titre.

A Prozone phenomenon may be observed: This is the inhibition of agglutination with low titers and the presence of agglutination with high titers, e.g. negative with 1/50 and 1/100 dilutions and positive with 1/400 and 1/800 dilution.

This phenomenon is due to the formation of blocking antibodies which are IgA antibodies interfering with agglutination with IgG or IgM. They appear in the chronic stage and may persist for years. They are detected by Coomb's antiglobulin test by adding rabbit antihuman globulin.

The sera of a certain proportion of the population may agglutinate Brucella suspension in low titers, this may be due to past infection or a latent infection.

C) Complement fixation test may be used

- a) To confirm agglutination test.
- b) In cases where agglutination test is negative.

D) 2-Mercaptoethanol test

Addition of this reagent to the patient's serum destroys IgM leaving IgG for agglutination. It is useful in chronic active disease.

E) Serum Brucella specific IgM and IgG by ELISA

F) Brucellin test

It is an allergic skin test produced by the intradermal injection of:

- a) A culture filtrate of Brucella.
- b) Brucella extract (brucellin).
- c) Purified protein.

The reaction resembles the tuberculin reaction, it gives rise to an edematous indurated area of at least 5 mm after 48 hours.

Treatment:

- 1) By the simultaneous administration of streptomycin and tetracycline for three weeks. If the doses are not adequate the condition passes into the chronic state which responds less to treatment because of their intracellular location.
- 2) Oral doxycycline and rifampin for six weeks.