ZOONOSIS

Genus Yersinia

Genus Yersinia includes 11 species but the main medical importance for human have *Y.pestis* (**causative agent of plague**), *Y.pseudotuberculosis* (causative agent of pseudotuberculosis), *Y.enterocolitica* (causative agent of intestinal yersiniosis).

Morphology. Yersinia are short, ovoid, gram-negative rods, non-motile, non-sporeforming surrounded by slime layer. They show bipolar staining (more intensive on the ends).

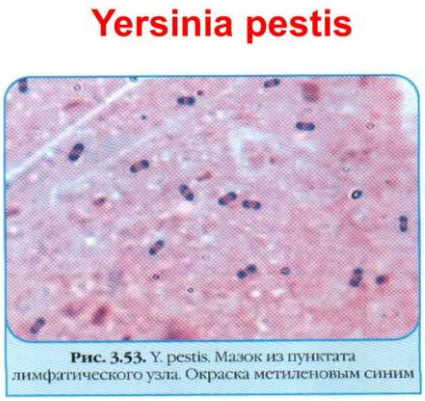


Рис. 3.53. Y. pestis. Мазок из пунктата лимфатического узла. Окраска метиленовым синим

Culture properties. *Y.pestis* are facultative anaerobes, grow readily on common media, but growth is better in the presence of hemolysed blood. Optimum temperature for growth is 27°C and optimum pH is 7.2. On the liquid media *Y.pestis* form delicate film which hangs down from the surface into the broth resembling *stalactites.* On the MPA they form dense colonies with yellow-turbid center and a thin transparent peripheral edge that resembles lace of handkerchiefs.

***Y.pestis*on blood agar**

Fermentative properties. *Y.pestis* ferment glucose, maltose, arabinose and mannitol with the production of acid, don't ferment lactose, sucrose and rhamnose. Indole is not produced. MR is positive, VP and citrate are negative, catalase and aesculin are positive, oxidase and urease are negative. Gelatin is not liquefied.

Resistance. *Y.pestis*are easily destroyed by exposure to heat, sunlight, drying and chemical disinfectants. They are destroyed by heat at 55°C or by phenol in 15 minutes. *Y.pestis* remain viable for long periods in cold, moist environments. All strains are lysed by a specific antiplague bacteriophage at 22°C.

Antigenic structure. The antigenic structure is complex. At least 20 antigens have been detected by gel diffusion and biochemical analysis. Yersinia is facultative parasite, ability to reproduce in the macrophages is mediated by V and W antigens. They inhibit phagocytosis and intracellular killing of the bacillus. Production of V and W antigens is plasmid mediated. A heat labile protein envelope antigen (FractionI or F-I) is best formed in cultures incubated at 37°C. It inhibits phagocytosis and is generally present only in virulent strains.*Y.pestis*are antigenically homogeneous and serotypes do not exist.

Virulent factors. Virulent factors include protein envelope antigen (F-I),V and W antigens, bacteriocin (PesticinI), coagulase and fibrinolysin. Pesticin inhibits *E.coli*, *Y.pseudotuberculosis* and *Y.enterocolitica*. Virulence also appears to be associated with an unidentified surface component which absorbs hemin and basic aromatic dyes in culture media to form coloured colonies. Virulence has also been associated with the ability for purine synthesis.

It produces two classes of toxin: endotoxin which is lipopolysaccharide, and exotoxin (murine toxin) which is protein in nature and possesses properties of both exotoxin and endotoxin. It produces beta-adrenergic blockade and is cardiotoxic and hepatotoxic. Plague toxins produce local edema and necrosis with systemic effects on the peripheral vascular system and liver.

Epidemiology. Plague is worldwide in distribution, with most of the human cases reported from developing countries. Plague is a zoonotic disease. The plague bacillus is naturally parasitic in rodents. Survival of the bacillus in nature depends on the flea-rodent interaction, and human infection does not contribute to the bacteria's persistence in nature. Infection is transmitted among rodents and to humans by the bite of an infected rat flea. Human-to-human transmission is rare except during epidemics of pneumonic plague.

Plague is the disease known in the middle ages as the «black death». This is because it frequently leads to gangrene and blackening of various parts of the body. Plague has caused large-scale epidemics, thereby changing the course of history in many nations. The first pandemic was believed to have started in Africa and killed 100 million people over a span of 60 years. In the early twentieth century, plague epidemics accounted for about 10 million deaths in India.

Pathogenicity. Human is infected by flea bites when bacteria are inoculated into the body, or by inhalation of aerosols containing bacilli generated from exhaled breath of pneumonic plague patients. The bulk of non-capsular organisms are phagocytized and destroyed by neutrophils. However, few organisms are taken up by histiocytes which are unable to kill them and allow them to resynthesize their capsule and multiply. The encapsulated organisms, when they are released from histiocytes, are resistant to phagocytosis and killing by neutrophils.

The resulting infection spreads to the draining lymph nodes which become hot, swollen, tender and hemorrhagic giving rise to the characteristic black buboes whence the name of the disease, bubonic plague is derived. Incubation period is 2 to 8 days. Within hours the organism spreads into the spleen, liver and lungs. Organisms enter blood and can cause septicemia. There may be hemorrhages from skin and mucous membrane.

Pneumonic type is spread through droplet infection. The incubation period is 1 to 4 days. Bacilli spread through lymphatics causing hemorrhagic pneumonia.

Immunity. Immunity after past infection of varying duration and intensity, is provided mainly by cellular immune response.

Microbiological diagnosis. Bacteriological, microscopic, biological, serological methods are used for plaque diagnosis. All studies are conducted in special laboratories that operate according to the instructions "On mode of operation of anti-plague institutions". Specimens for investigation are punctates from the bubo, sputum, carbuncles and ulcers excretion, blood, urine, vomiting, cadaverous material. Material is inoculated on the nutrient media (MPA, blood agar, MacConckey agar). Isolated pure culture is tested by biochemical reactions.

Species	**Oxidase**	**Indol**	**Urease**	**VP**	**MR**	**Motility at 22°**	**Maltose**	**Sucrose**	**Ornithine decarboxylase**
Y.pestis	-	-	-	-	+	-	A	-	-
Y.pseudo-tuberculosis	-	-	+	-	+	+	A	-	-
Y.enteroco-litica	-	-/+	+	-	+	+	A	A	+

The organisms can be identified with a fluorescent antibody staining technique, and the epidemiology of the outbreak can be traced by bacteriophage typing.

Serological tests are sometimes useful in diagnosis. Antibodies to the F-l antigen may be detected by agglutination or complement fixation tests. The latter test may be used also for detecting the antigen in tissues. The passive hemagglutination test, using tanned erythrocytes coated with the F-l antigen or murine toxin is useful for identifying plague foci, as the test remains positive for several years after the recovery, from plague. Thermoprecipitation test is used to examine the putrefied tissue for detection of antigen.

Biological method is used to verify the diagnosis. Guinea pig or albino rats are infected subcutaneously with isolated culture of plague bacilli. Animal dies within 2-5 days. An autopsy shows necrosis, edema with involvement of regional lymph nodes, spleen enlarged and congested. In septicemia, bacilli may be demonstrated by drawing smear from local lesionised lymph nodes, splenic pulp and heart blood.

Treatment: Tetracycline doxycycline, chloramphenicol, cotrimaxozole plus gentamycin or kanamycin or streptomycinare effective.

Prophylaxis. In the prevention of domestic plague, general measures such as control of fleas and rodents are of great importance. All patients with suspected plague should be isolated. Specific protection may be provided by vaccines. Two types of vaccines have been in use - killed and live attenuated vaccines EV. A formalin killed vaccine is mainly indicated for travellers to hyperendemic areas and for persons at special higher risk. Live plague vaccines cause severe reactions and are not in use now.

Y.pseudotuberculosis

Morphology. *Y.pseudotuberculosis* are short, ovoid, gram-negative rods, motile.

Culture properties. It grows readily on common media. Optimum temperature for growth is 22-28°C. On the liquid media it forms delicate film.

Fermentative properties. *Y.pseudotuberculosis* ferment rhamnose, don't ferment sucrose. Indole is not produced. It is VP and citrate negative.

Antigenic structure. *Y.pseudotuberculosis have O- and H-antigens.* The strains of Y. pseudotuberculosis are of 6 serological types (serotypes 1-6) based on highly specific somatic antigens.

Epidemiology. Pathogenicity. The sources of infection are animals and birds. Pseudotuberculosis is a zoonosis transmissible from infected animals to human either through skin contact with contaminated water or through the consumption of contaminated vegetables or other footis. It occasionally results in a severe, generalized disease with a high fatality rate. More commonly it gives rise to acute mesenteric adenitis, simulating acute or subacute appendicitis (right iliac fossa syndrome), sometimes with the added complication of erythema nod000.osum, usually in young males.

Microbiological diagnosis. Specimens for investigation are urine, blood, mucus. The diagnosis is confirmed by isolating the organism from material and by demonstrating antibodies in the patient's serum during the acute phase of the infection with agglutination and indirect hemagglutination tests. ELISA is used to detect IgM in acute phase of disease.

Treatment. Chloramphenicol and cephalosporins are drugs for choice. In severe forms of the disease combination of one of these antibiotics with aminoglycosidesis possible.

Prophylaxis. Nonspecific prevention involves continuoussanitary control of water supply, mode processing and storage products, fight with rodent. Specific prophylaxis is not developed.

Y. enterocolitica.

Y. enterocolitica are gram-negative rods, motile, non-sporeforming. The biological properties differ little from the causative agents of pseudotuberculosis. They are aerobe and facultative anaerobe. Optimum temperature is 22-29°C; they multiplyat 4°C (which constitutes a hazard when contaminated food is refrigerated). They grow slowly on artificial media; on blood agar form non-haemolytic, smooth, translucent colonies. Selective media or enrichment techniques are necessary for isolation from faecal specimens.

Y. enterocolitica differs from *Y. pseudotuberculosis* in its ability to ferment sucrose, sorbitol, cellobiose but not salicin, and in being ornithine decarboxylase positive. On the basis of variations in certain biochemical tests *Y. enterocolitica* may be divided into six different biotypes.

Y. enterocolitica is divided into a large number of serotypes depending on 34 different O-antigen factors and 19 H-antigens. Serotypes 3 and 9 are associated withthe majority of human infections, especially in Europe.

Pathogenic serotypes produce a heat-stable enterotoxin similar to that produced by enterotoxigenic E.coli. Because the toxin is not produced at temperatures exceeding 30°C it may be developed by organisms growing in contaminated food stored at low temperatures. This may explain the occurrence of some cases of food poisoning from which no causative organism has been isolated. Toxigenic strains do not ferment rhamnose.

Y. enterocolitica infections are not considered to be true zoonoses. Human infections probably occur from ingestion or contact. Family and other small outbreaks suggest that person to person transmission occurs. Intestinal yersiniosis often manifests as gastrointestinal forms, rarely in a generalized forms. Sometimes, generalized form is accompanied by the development of specific hepatitis.

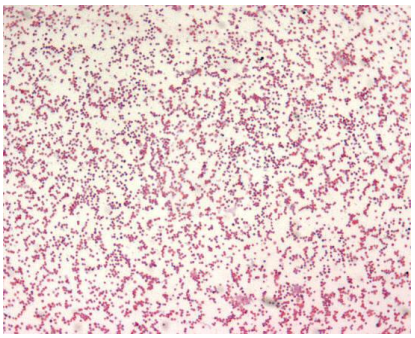
For microbiological diagnosis bacteriological method of investigation is used. Materials for investigation are feces, urine, blood. The diagnosis is confirmed by isolating the organism from material and by demonstrating antibodies in the patient's serum by indirect hemagglutination tests.A rising titer of 160 and over is significant.

Chloramphenicol, cephalosporins, aminoglycosides can be recommended for treatment of middle and severe forms of the diseases. Nonspecific prevention involves continuous sanitary control of water supply, mode processing and storage products. Specific prophylaxis is not developed.

Genus Francisella

Genus Francisella belongs to the family Francisellaceae and includes 2 species: *F.novicida* and *F.tularensis*. *F.tularensis* are human pathogens, which cause zooanthroponosis **disease tularemia.**

Morphology. *F.tularensis* are bipolar-staining coccobacillus, capsulated, non-motile and non-sporeforming. Pleomorphic forms can be found, especially in cultivation or in infected tissue.



***F.tularensis* Gram staining**

Culture properties. *F.tularensis* are obligate aerobe that have an optimum growth temperature of 37C. They require a complex medium confining blood, tissue extracts and cystine. Minute droplet-like colonies develop after 48 hours. Trypticase soy broth, supplemented with 0,1% cysteine, a modified Mueller–Hinton broth can be used for the liquid cultivation of Francisella. In the liquid media they form surface pellicle.

*F.tularensis*colonies

Fermentative properties. *F.tularensis* ferment maltose, glucose, mannose, levulose, glycerin with acid formation. They don't produce indol, and they are urease negative.

Antigenic structure. *F.tularensis* contain somatic O-antigen and surface Vi-antigen. During the cultivation, *F.tularensis* tend to dissociate in the R-form with the loss capsules, Vi-antigen and standard antigenic properties.

Virulent factors. The mechanisms involved in causing the symptoms of tularemia are poorly understood. *F.tularensis* don't produce exotoxin. The species are intracellular parasites. They produce enzymes that inhibit action of phagocytes leading to non-complete phagocytosis. Outer membrane antigen has properties of endotoxin.

Resistance. *F.tularensis* are killed by moist heat at 55-60°C in 10 min. They may remain viable for many years in cultures kept at 10°C, and in humid soil and water for 30 and 90 days respectively.

Epidemiology. Due to its low infectious dose, ease of spread by aerosol, and high virulence, F. tularensis is classified as a Tier 1 Select Agent by the U.S. government, along with other potential agents of bioterrorism such as Yersinia pestis, Bacillus anthracis and Ebola virus. The reservoir of causative agent is rodents. Housekeepers and field mice, water rats, muskrats, rabbits, squirrels are the sources of infection. Tularemia is caused by contact with infected animals or vectors such as ticks, mosquitos, and deer flies, alimentary, dust-airborne ways.

Pathogenesis. The most common tularemia occurs via skin contact, yielding an ulceroglandular form of the disease. Inhalation of bacteria leads to the potentially lethal pneumonic tularemia. No case of tularemia has been shown to be initiated by human-to-human transmission.Regardless of the entry route, F. tularensis can disseminate from the initial infection site to the lungs where it can cause respiratory tularemia, the most severe form of the disease.

The portal of entry for F.tularensis is skin, mucous membrane of upper respiratory tract, gastro-intestinal tract, eyes. They spread from the initial site of infection through lymphatic channels to the local lymph glands, multiply there causing local inflammation. Then they overcome lymphatic barrier, enter into the bloodstream and disseminate throughout the body in the liver, spleen, lungs, causing secondary inflammation. Forms of disease are: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, typhoidal, septic.

Immunity. Immunity after the disease remains stable, mostly lifelong.

Microbiological diagnosis. Exudate from lymph node, pus, pharyngeal washings, sputum specimens, gastric aspirates, blood may be used for investigation. F. tularensis may be identified through direct examination of secretions, exudates, or biopsy specimens using Gram stain, direct fluorescent antibody, or immunohistochemical stains. Microscopic demonstration of F. tularensis using fluorescent-labeled antibodies is a rapid diagnostic procedure performed in designated reference laboratories. Growth of *F. tularensis* in culture is the definitive mean of confirming the diagnosis. Sometimes growth of the bacteria can be delayed up to 10 days.

Biological method includes inoculate of investigated material into guinea-pigs and mice. The liver and spleen of the postmortem animals are cultivated on special medium for isolation of pure culture and its identification. F.tularensis are identified by morphological, culture, biochemical properties and by agglutination test with tularemia diagnosticum. Diagnostic titer of agglutination test is 1:100. Other serological tests (CFT, IHT) also may be used. The earliest and highly specific test is a skin-allergic test with tularin, which is positive for 3-5 day illness.

Treatment. Aminoglycosides, tetracyclines, chloramphenicol are used for treatment.

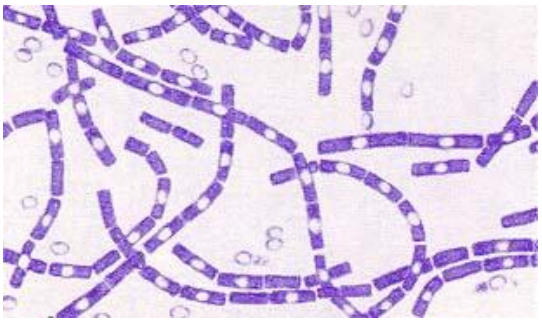
Prophylaxis. Live attenuated vaccine is used for specific prophylaxis in areas where tularemia is considered endemic. Nonspecific prophylaxis consists of deratization measures in epizootic foci.

Bacillaceae family

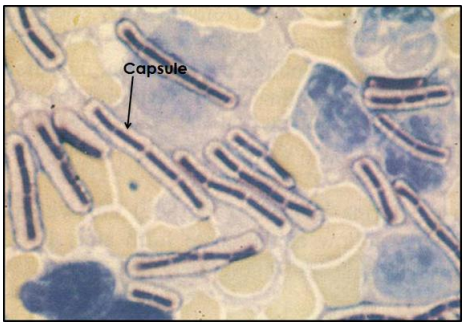
Members of the family Bacillaceae genus Bacillus are widely represented in nature. Pathogenic for human is *B. anthracis*, opportunistics are other microorganisms – *B. cereus*, *B.subtilis*, *B.mesentericus*, *B.megaterium* and others.

Bacillus anthracis

Morphology. *B.anthraci* are gram-positive, non-motile, square-ended rod-shaped bacteria. They are arranged in chains in culture, but single or in pairs in animal's blood. It is one of few bacteria known to synthesize a protein capsule. Capsules are formed in the body, but in culture only on media with serum or bicarbonate in the presence of excess CO2. Outside of the body they form oval spores located centrally in a non-swollen sporangium. Bacillus anthracis spores in particular are highly resistent, surviving extremes of temperature, low-nutrient environments, and harsh chemical treatment over decades or centuries.

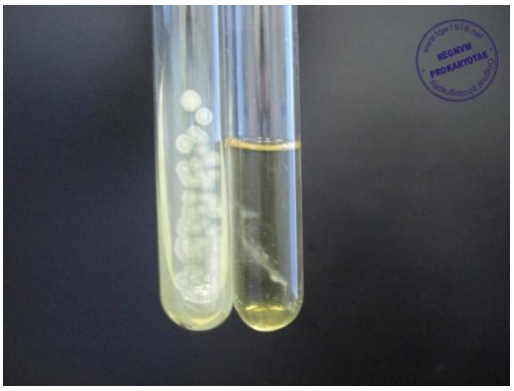


***B.anthracis* spores**



***B.anthracis* capsules**

Culture properties. *B.anthracis* are aerobic. They grow in an ordinary nutrient medium at temperature 35-37°C. On the MPA they form rough R-shaped colonies: raised, dull opaque, grayish white coloniesresemblefrosted glass appearance. Edge of the colony is composed of interlacing chains of bacilli looking like matted hair - medusa head appearance.Colonies on blood agar produce very slight hemolysis. On MPA with penicillin, vegetative forms in the chains transform in the spheroplasts, that in the smears resemble necklace of pearls.In MPB, growth develops as floccular sediment resembling cotton wool.



Fermentative properties.*B.anthracis* are fermented glucose, maltose, sucrose and other carbohydrates with acid, hydrolysed starch. They produce protease, lipase, catalase, peroxidase, gelatinase. *B.anthracis* form ammonia, hydrogen sulfide, reducing nitrates to nitrites, slowly fermented milk, have weak hemolytic, lecithinase and phosphatase activities. In a gelatin stab, there is growth down the stab line with lateral spikes that are the longest near the surface, giving

the *"inverted fir tree*" appearance; liquefaction is late and starts at the surface. Coagulated serum is partially liquefied by *B.anthracis*.

Antigenic properties. There are three main antigens observed in serological tests: the exotoxin complex (protein), the capsular polypeptide and a somatic polysaccharide. Exotoxin complex antigen is protective. Synthesis of capsular and exotoxin antigens are determined by plasmids. Plasmids loss leads to loss of virulence of bacteria.

Virulent factors. The virulence is dependent upon the presence of the capsule (antiphagocytic activity) and production of the toxin.The toxin is a complex of three fractions: the edema factor, the protective antigen factor and the lethal factor. Protective antigen is the fraction which binds to the receptors on the target cell surface. Edema factor is an adenyl cyclase which is activated only inside the target cells, leading to intracellular accumulation of cyclic AMP and is responsible for the edema of tissues. Entry of lethal factor into the target cell causes cell death and edema of the lungs.These three factors are not toxic individually but the whole complex produces local edema and generalised shock.

Resistance. In the dry state or in soil the spores may survive for many years. The vegetative bacilli are killed at 60°C in 30 min with moist heat, and the spores - at 100°C in 20 min. With dry heat the spores are killed at 150°C in 60 min. The spores are also killed by 4% (w/v) formaldehyde or 4% (w/v) potassium permanganate in a few minutes.

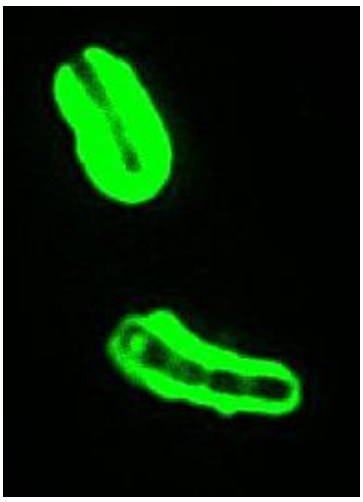
Epidemiology. Anthrax is a zoonosis. Cattle, sheep, goats, pigs and other herbivores are naturally affected. Mice, guinea pigs, rabbits, hamsters and monkeys are susceptible to experimental infection. Reservoir of pathogen is the soil. Animals are infected by ingestion of the spores present in the soil. The most often contact mechanism of infection occurs, less often alimentary and respiratory ways of transmission.

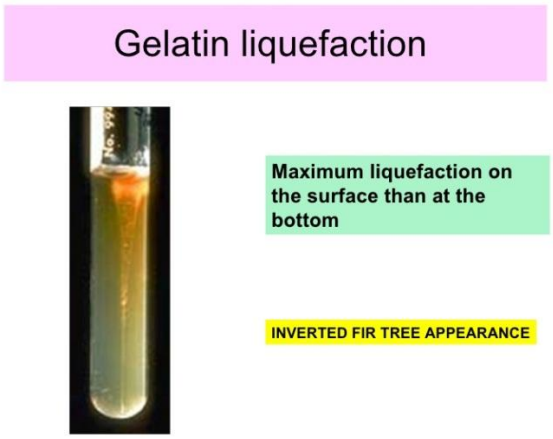
Pathogenesis. The portal of entry is skin, less often mucus membrane of respiratory and GIT. The portal of entry is skin, less often mucus membrane of respiratory and GIT.

Three clinical forms of anthrax disease are recognized based on their form of infection.*Cutaneous* form is the most common (95%), causes a localized inflammatory black necrotic lesion (eschar). *Pulmonary form* is called the "wool sorter's disease" because it used to be common in workers in wool factories, due to the inhalation of dust from infected wool. This is a hemorrhagic pneumonia with a high fatality rate and characterized by sudden massive chest edema followed by cardiovascular shock. *Gastrointestinal form* is rare but also fatal (causes death to 25%) type results from ingestion of spores. All types lead to fatal septicemia.

Immunity. Immunity after the disease is strong and long-lasting, antibacterial and antitoxic. Cell-mediated hypersensitivity is formed after the disease and may be diagnosed with skin test.

Microbiological diagnosis. Material for investigation is exudate from carbuncule, mucus, feces, urine, blood. Microbiological diagnosis is carried out in compliance with safety regulations as at especially dangerous infections. At microscopic investigation smears are stained by Gram's and by Romanovskyi-Giemsa's methods. Immunofluorescent microscopy can confirm the identification and is used as rapid specific diagnostic test.





IF stain of *B.anthracis* capsule

Investigated material is inoculated onto the nutrient agar and blood agar, and also in broth. Isolated pure culture is identified by morphological, culture, biochemical properties.

Table Differential signs of Bacillus

Signs	***B.anthracis***	***B.anthracoides***	***B.subtilis***
Motility	-	weak	+
Capsule	+	-	-
Blood agar	-	hemolysis	hemolysis
Litmus milk	reddening	bluish	bluish

Biological method is used to isolate the anthrax bacillus from contaminated tissues by applying them over the shaven skin of a guinea pig. The animal dies in 24-72 h, showing a local, gelatinous, hemorrhagic edema at the site of inoculation. The capsulated bacilli are found in large numbers in the local lesion, heart blood and spleen.

Serological diagnosis is used in cases if is not possible to isolate B. anthracis from patients. ELISA, CFT, IF IHAT, precipitation tests are used for detection of antigen. *Ascoli's thermoprecipitin tes*t is used for demonstration of the anthrax antigen in tissue extracts.

The allergic test with anthracin (a purified anthrax allergen) is employed when a retrospective diagnosis is required in cases which have yielded negative results with microscopic and bacteriological investigation.

Treatment. Penicillin, tetracycline, fluoroquinolones and streptomycin are drugs for treatment.

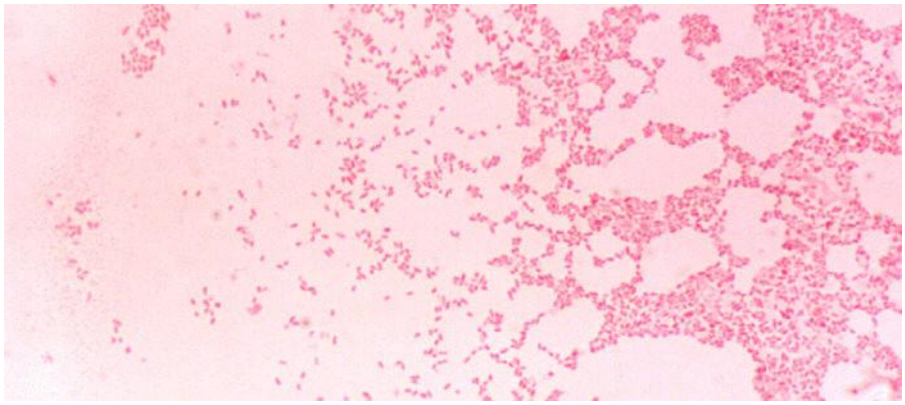
Prophylaxis. Prevention of human anthrax is mainly by general methods such as improvement of factory hygiene and proper sterilisation of animal products like hides and wool. Carcasses of animals suspected to have died of anthrax are buried deep in quicklime or cremated to prevent soil contamination.

Specific prophylaxis is mediated by alive *CTI* vaccine.

Genus Brucella

Brucella is a genus of gram-negative bacteria of family Brucellaceae. In modern taxonomy 6 independent Brucella species are: *B.melitensis, B.abortus, B.suis, B.neotome, B.ovis, B.canis*. The different species of Brucella are genetically very similar, although each has a slightly different host specificity. Hence, the NCBI taxonomy includes most Brucella species under the *Brucella melitensis* (type species).The species are intracellular parasites of humans and animals producing characteristic infections, particularly of the reticuloendothelial and reproductive systems.

Morphology. Brucellae are small non-motile, non-sporeforming, non-capsulated non-acid fast gram-negative coccobacilli. They are arranged singly, rare in pairs, short chains or small clusters. Bipolar staining can occur, especially in old cultures.



***B.melitensis* Gram staining**

Culture properties. Brucellae are strictly aerobic organisms but many strains of B. abortus require the addition of 5-10% CO_2. Temperature range is 20-40°C, optimum is 37°C. They grow rather slowly on ordinary nutrient media. Growth is improved by serum, blood or liver extract. Prolonged incubation (up to six weeks) may be required, as they are slow-growing. On solid media colonies are small, smooth, transparent, low convex with an entire edge with bluish-gray pigmentresembles droplets of dew. In liquid media uniform turbidity with follow sedimentation is forming.

***B.melitensis* colonies**

Fermentative properties. The organisms have little or no fermentative action on carbohydrates in conventional media. They are catalase positive and usually oxidase positive, reduce nitrates, and hydrolyze urea to a variable extent.Indole is not produced, and MR and VP tests are negative. Classification into biotypes and biovars is based on CO_2 requirement, H_2S

production, fermentation of sugars, inhibition by bacteriostatic dyes, agglutination by monospecific sera to A, M, and R antigens, and lysis by specific bacteriophage.

Test	*B.melitensis*	*B.abortus*	*B.suis*	*B.neotome*	*B.ovis*	*B.canis*
need CO_2	-	+	-	-	+	-
production of H_2S	-	+	±	+	-	-
Glucose	+	+	+	+	-	+
Arabinose	-	+	±	+	-	-
Galactose	-	+	±	+	-	-
ribose	-	+	+	+	-	+
xylose	-	-	+	+	-	-
ornithine	-	-	+	-	-	+
A-serum	+	+	+	+	-	-
M-serum	+	+	-	-	-	-
Anti-R-serum	-	-	-	-	+	+

Antigenic structure. Group-specific O-somatic antigen and species-specific A, M, and R antigens are distinguished in Brucella spp.Various Brucella species contain a set of A-M antigens in different proportion.

Virulent factors. Brucella produce hyaluronidase that promotes their invasion. The species are intracellular parasites, because, getting into macrophages, they secrete substances that inhibit phagosome-lysosome fusion. Pathological changes in the body occur under the influence of endotoxins. *B. melitensis* is the most pathogenic.

Resistance. Brucellae are destroyed by heat at 60 °C in 10 minutes and by 1% phenol in 15 minutes. They may survive in soil and manure for several weeks. They remain viable for 10 days in refrigerated milk, one month in ice cream, four months in butter,many weeks in meat and for varying periods in cheese depending on its pH. They are sensitive to direct sunlight and acid, and tend to die in buttermilk.

Epidemiology. Brucellosis is an antropozoonotic infectious disease. The sources of human infection are goats, sheep, cattle, buffaloes, and swine. *B.melitensis* is predominant in goats and sheep, *B.abortus* is in cattle and *B.suis* is in swine.The ways of infection transmission are by ingestion, contact, inhalation or accidental inoculation. Person to person spread does not ordinarily occur.The most important vehicle of infection is raw milk. Contact infection is especially important as an occupational risk in veterinarians, butchers, and animal handlers. Respiratory transmission is by inhalation of dried material of animal origin such as dust from wool. Infection by inhalation is a serious risk in laboratory workers handling brucella.

Pathogenesis. The brucellae spread from the initial site of infection through lymphatic channels to the local lymph glands, in the cells of which they multiply. Incomplete phagocytosis at brucellosis turns macrophages in the inner reservoir of the pathogen, which are inaccessible to chemotherapeutic agents and specific antibodies. They then spill over into the bloodstream and are disseminated throughout the body that lead to bacteremia and inflammation in the liver, spleen, bone marrow, endocardium, synovial membrane of joints. Herewith the agent is removed from the blood. After some time the process of the disease generalization is repeated, accompanied by acute exacerbation of clinical manifestations.

Acute brucellosis is associated with prolonged bacteremia and irregular fever. The symptoms are varied, consisting of muscular pains and arthralgia, asthmatic attacks, exhaustion, anorexia, constipation, depression, nervous irritability and chills. Chronic brucellosis, which may be nonbacteremic, is a low grade infection with periodic exacerbations. The symptoms are generally related to a state of hypersensitivity, the common clinical manifestations are sweating, lassitude and joint pains, with minimal or no pyrexia. The illness lasts for years.

Immunity. Immunity in brucellosis is weak, mainly cell mediated, non-sterile. Activated macrophages can kill the bacteria. This is probably the most important mechanism in recovery and immunity in brucellosis.

Microbiological diagnosis. Bacteriological, serological, biological, allergic and PCR examinations are usually essential for confirmation of the diagnosis. Bacteriological and biological methods of diagnosis are performed in the laboratory of especially dangerous infections with compliance of strengthened safety rules. Blood, punctate of bone marrow and lymph nodes, urine, milk are materials for the investigation. Blood cultures are best performed on samples collected during the pyrexial phase, preferably while the temperature is rising. Blood (20 ml) is inoculated into a bottle of trypticase soy broth and incubated at 37 °C under 5-10% CO2. Subcultures are made on solid media every 3-5 days, beginning on the fourth day within the month.

For serological diagnosis the standard tube-agglutination test (*Wright's test*) is the most widelyused. This is a tube agglutination test in which equal volumes of serial dilutions of the patient's serum and the standardised antigen (a killed suspension of a standard strain of *B.abortus*) are mixed and incubated at 37 °C for 24 hrs. A titre of 200 or more is considered significant.

Simplified and accelerated version of agglutination reaction with brucella diagnosticum is *Heddelson's test*, which is carried out on the glass plates. In this test, blood taken from the finger performing the reaction in a few minutes. Heddlson's reaction is proposed to identify the persons to be examined for brucellosis, with mass screening for epidemiological indications. Other serological tests (CFT, IHA) are less informative than the Wright's reaction and are not essential.

Burnet skin allergic test with brucella antigens ('brucellins') is useful in diagnosis of chronic brucellosis. Allergic test (delayed hypersensitivity type) is positive from 15-20 days of the disease and may remain positive for years.

Treatment. Because Brucella survives within phagocytes, antibiotics with good intracellular penetration are recommended. The treatment of choice is doxycycline and rifampin for 6 weeks. Single-drug therapy is not recommended because of the high like-lihood of relapse. Doxycycline combined with intramuscular streptomycin or gentamicin (5 mg/kg) are useful alternatives. For children, trimethoprim sulfamethoxazole (10 to 12 mg/kg of the trimethoprim component daily, divided into two doses) and rifampin (20 mg//kg daily) are recommended. In cases of meningitis or endocarditis, a three-drug regimen consisting of doxycycline, rifampin, and trimethoprim–sulfamethoxazole has been used. Therapy for these diseases must be prolonged (several months to more than 1 year). In patients with endocarditis, replacement of the infected valve is usually required for cure.

Killed vaccine may be used for treatment of chronic infection.

Prophylaxis. Specific prevention of brucellosis is conducted by alive vaccine from avirulent strain of *B.abortus*, which provides the formation of cross immunity to other types of pathogen.Nonspecific prophylaxis includes checking brucellosis in dairy animals, pasteurisation of milk.