

# **Regionally relevant pathogens**

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# Anthrax

#### Summary

- Anthrax is caused by a large Gram-positive bacterium, Bacillus anthracis
- Bacterium can survive adverse environmental conditions as a resistant spore
- Can be used as a biological weapon
- There is no human-to-human transmission
- Pathology caused by powerful exotoxins
- Cutaneous anthrax: skin ulcers with oedema
- Respiratory anthrax: fulminant mediastinitis / pneumonia, meningitis, septicaemia
- Treatment with penicillin, ciprofloxacin, rifampicin, clindamycin
- Neutralisation toxin with antitoxin, e.g. raxibacumab or analogues

## General

Anthrax is a widespread zoonotic infectious disease caused by a **large Gram-positive rod-shaped** bacterium: *Bacillus anthracis*. Anthrax is usually a disease of **herbivores**. The animals are infected by grazing in an area contaminated with bacterial spores. Mortality in these animals is high and the carcasses will in turn contaminate the soil. This animal disease also affects man. People die not so much from the invasion of this pathogen but from the **toxins** that are secreted.

The causative agent of anthrax was identified by French biologist Casimir-Joseph Davaine in 1863 and by German bacteriologist Robert Koch, who isolated the organism in pure culture in 1876.



### Toxin

The bacterium is surrounded by a polypeptide capsule (polyglutamic acid) that protects the pathogen against phagocytosis. As in other toxin-dependent diseases caused by Grampositive bacteria such as tetanus or diphtheria, the pathogenesis of anthrax is attributable in the first place to **exotoxins** that are produced. Strains that cannot produce toxins are avirulent. The principal virulence factors of *B. anthracis* are coded on two **plasmids**; one involved in the synthesis of the capsule and the other coding the exotoxins.

The vegetative pathogen releases toxins that have a complex action. The exotoxins are binary and consist of a **B (binding) protein that is necessary for cell penetration** and an **A (active) protein that causes metabolic dysfunction**. There are three proteins: PA (protective antigen), LF (lethal factor) and EF (oedema factor).

LF is a zinc metalloprotease which kills cells by proteolytic cleavage of several members of the MAP kinase signal transduction pathway. EF is a calmodulin-dependent adenylate cyclase which catalyses the conversion of ATP to cAMP, causing an elevation in cAMP levels. This leads to pronounced oedema, inhibition of neutrophils and monocytes.

#### **Anthrax spores**

In certain circumstances *B. anthracis* can form an **endospore**. Spores like this are **very resistant to unfavourable environmental conditions**. The pathogen survives as a spore in the soil for many years, but seemingly less easily in acid soil than neutral soil.

#### Anthrax spore survival

This long survival was shown very clearly by experiments in the Second World War when Gruinard Island to the north-west of Scotland was deliberately contaminated with the pathogen in order to establish the effects on experimental animals such as sheep. Many years later viable spores of the bacterium were still found in the soil. This required a very aggressive decontamination of the whole of the island in 1986.



In April 1979 there was a notorious accident in Sverdlovsk (now Ekaterinburg) in Russia, in which 66 people died from inhalational anthrax. It is now certain that the cause was an accident in a biological weapons installation of the Russian BioPreparat Programme. About 10 kg of anthrax spores (4 different strains) were released because someone failed to replace a filter on an air vent. People were infected up to a distance of 4 km away from the installation. There were even cases in animals 50 km further away. All the cases occurred in a period of 6 weeks after the incident.

In the period 1979-80 there was an epizootic among cattle in Zimbabwe with about 10,000 infections (epizootic = "epidemic in animals"). Human cases were generally limited to cutaneous anthrax.

### **Clinical aspects**

#### **Cutaneous anthrax**

If someone has contact with animal fur or skin in which there are anthrax bacteria, the skin can become infected. Infection can also follow a bite by an infected horsefly (mechanical transmission of the pathogen).

After a **short incubation period of 2 to 3 days**, a **small red skin wheal** occurs at the inoculation site. This can itch at first. Over the course of the next week vesicles form around the central lesion. Occasionally there are atypical cases without vesicles. A central painless ulceration follows. The ulcer is dry, with minimal or no pus. There is often a black crust, hence the name "anthrax" = charcoal. The ulcer is surrounded by red gelatinous local oedema, which sometimes becomes massive (e.g. lesions in the face / neck). Regional lymphadenopathy with lymphangitis and moderate fever can occur, but often the patient is afebrile. The pathogens can multiply in the lymph nodes. The regional lymph nodes are often painful. Superinfection by pyogenic pathogens is rare. There is no peripheral leucocytosis. The skin lesion heals slowly (2-6 weeks) in more than 90% of cases but in rarely there is progression of the infection, with systemic involvement. Without antibiotics mortality can be as high as 20 percent.



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# **Cutaneous anthrax**

#### Summary

- The lesion can be similar to the consequences of a bite by a *Loxosceles* spider. It is usually easy to distinguish from orf, since there is no oedema in this viral infection.
- Cowpox generally leads to less oedema.
- Herpes simplex can resemble cutaneous anthrax.
- Cat-scratch disease has a slower course.
- Cutaneous tularaemia can occur in similar circumstances (contact with an infected animal).
- A pyogenic lesion such as a furuncle is usually caused by *Streptococcus pyogenes* or *Staphylococcus aureus* suppurates and is painful.
- Ecthyma gangrenosum may occur in patients with neutropenia and/or *Pseudomonas aeruginosa* bacteraemia.
- Cutaneous leishmaniasis develops much more slowly and is not so painful

### Diagnosis

A **Gram stain of a smear of the lesion** shows the typical large Gram-positive rods (1-1.5 x 4-10 µm). The bacterium is noticeably larger than most other pathogens. *Bacillus anthracis* is morphological very similar to *Bacillus cereus* and *B. subtilis.* These latter two pathogens do not however cause any lesions that may be confused with anthrax. If a person is infected; spores are not produced during the disease. An alternative to Gram stain is polychrome methylene blue (M'Fadyean's stain). This stain is based on the use of an alkaline methylene blue solution in which progressive oxidative demethylation occurs on ageing. With this stain the bacterium is coloured blue-black. The **large size** and a somewhat **square, blocky appearance** are typical. A rose-coloured capsule can be seen with M'Fadyean's stain. A culture confirms the identity of the pathogen (wound culture, blood culture, CSF, biopsy). The bacterium grows easily under aerobic conditions on sheep blood agar. *Bacillus anthracis* 



forms typical large grey-white, tenacious, non-haemolytic colonies. If anthrax is suspected the lab should be notified as spores can form in a Petri dish, with risk for transmission in the lab. It is detected in a blood culture within 24 hours. It should be noted that the pathogen is Gram-positive in young cultures but can become Gram-variable afterwards. In aerobic cultures the pathogen soon loses its capsule. The absence of a capsule on for example sheep blood agar is therefore not an argument against *B. anthracis*. Other specific culture methods are necessary in order to demonstrate the capsule. Culturing in the presence of 5% CO2 on basal media with 0.8% NaHCO3 shows densely encapsulated bacteria, visible with India ink stain. The bacterium is not motile and can develop central or subterminal spores if the nutrients in the medium are exhausted. The bacterium is **usually** sensitive to penicillin, with a clear inhibition zone on an agar plate around the antibiotic disc. Serology (e.g. ELISA) can be carried out in order to detect antibodies against lethal toxin and oedema toxin but has **no place in acute diagnostics**. Serology is clearly less sensitive in cutaneous anthrax (67%) than in inhalational anthrax (94%). PCR and related techniques can be used for rapid identification. Using immunohistochemical techniques the pathogen, the capsule and polysaccharide cell wall antigens can be detected in tissue slices. There is a "Direct Fluorescent Antibody" (DFA) test that is used for rapid diagnosis of anthrax in exudates from skin lesions. The technique is not very sensitive for inhalational anthrax. Research into fast detection techniques of anthrax spores in micro-samples and dust clouds is ongoing, especially after 9/11 in the USA.







Cutaneous Anthrax. Copyright ITM

#### **Pulmonary anthrax**

If anthrax spores are **inhaled** ("woolsorters' disease"), after a short incubation period, high fever and dyspnoea occur. Once the bacteria have produced enough toxin (after 1-3 days), antibiotics are less effective. The primary lesion in inhalational anthrax is rarely in the nasal mucosa. For the first three days the symptoms are atypical; with fever, malaise, myalgia, a dry cough, chest pain, abdominal discomfort, nausea and vomiting. Then the disease develops dramatically. B. anthracis spores are phagocytosed by alveolar macrophages and transported to mediastinal lymph nodes. There they germinate, multiply, and release toxins, causing haemorrhagic necrosis of the thoracic lymph nodes draining the lungs, which results in a haemorrhagic mediastinitis and, in occasional cases, a necrotizing pneumonia. The organisms then become bloodborne, causing bacteraemia and in some cases meningitis. The clinical picture is that of a **fulminant pneumonia or mediastinitis** comparable with plague pneumonia (*Yersinia pestis*), pulmonary hantavirus, severe pulmonary leptospirosis, SARS, influenza or pulmonary tularaemia (*Francisella tularensis*).

A high fever, dyspnoea, stridor, cyanosis and shock characterise the course of the disease. Stridor is caused by extrinsic compression of the trachea by enlarged lymph nodes, mediastinal widening and subcutaneous emphysema of the chest and neck. Haemorrhagic necrosis of the hilar lymph nodes and mediastinitis follow. On a chest X-ray, **pleural fluid and a typical widening of the mediastinum** can be seen (DD of this important observation: post-surgical infection, rupture of an aortic aneurysm and contused chest trauma such as with deceleration lesions). Petechiae and splenomegaly occur. On a CT scan of the chest a widened mediastinum, pleural fluid and enlarged hilar lymph nodes are seen. These lymph nodes have about the same density as the aorta, which reflects the haemorrhagic-necrotic nature. **Mortality used to be very high** (almost 100%) but can be reduced to below 50% by starting aggressive antibiotic therapy quickly (see below) and raxibacumab.



### **Meningeal anthrax**

The cerebral membranes can be affected, leading to a black haemorrhagic discolouring of the meninges. Red blood cells and many neutrophils, as well as the bacterium itself are found in the CSF. This complication occurs with haematogenous dissemination in about **50% of inhalational** anthrax. Only the vegetative pathogens (not the spores) are found in the CSF. Mortality is 75% within the first 24 hours after presentation and overall survival only 6%.

#### **Gastrointestinal anthrax**

Gastrointestinal anthrax tends to occur in family clusters or point-source outbreaks. After eating food infected with anthrax (for example an animal that has died from anthrax), infection of the throat or intestines can follow. Gastrointestinal anthrax is characterised by fever, **ulcerative intestinal lesions** in the caecum or terminal ileum, bloody diarrhoea and the development of shock. Hematemesis can be caused by bleeding stomach ulcers. Haemorrhagic mesenteric lymphadenitis with prominent ascites can occur. There is high mortality. Intestinal anthrax is rare. Differential diagnosis includes campylobacteriosis and yersiniosis. Necrotic enteritis (infection with toxicogenic Clostridium perfringens) or pigbel might be considered in malnourished patients presenting with acute necrosis of the jejunum, or more rarely ileum, caecum or colon.

The bacterium rarely causes inflammation of the throat (oropharyngeal anthrax), which can resemble diphtheria or plague with oedema, tissue necrosis and lymphadenopathy. On draining, the pus has a notably foul-smelling odour. Occasional cases of anthrax have been reported in IV heroin users. In these cases the heroin was apparently mixed with infected diatomaceous earth, resulting in soft tissue infections with a similar clinical picture to gas gangrene and mimicking necrotising fasciitis. Other soil bacteria such as *Clostridium novyi* have also been found in the same group of patients.

## Treatment

For severe cases the **combination of several antibiotics which have complementary working mechanisms such as ciprofloxacin, rifampicin and clindamycin** has been suggested. With this combination therapy, mortality from pulmonary anthrax in the USA has



been reduced to 40%. Meropenem can be added if the meninges are involved and linezolid is favoured over clindamycin because it is likely to have better CNS penetration. Other fluoroquinolones and doxycycline can also be used. Because of inducible beta-lactamase activity, monotherapy with penicillin G, ampicillin or amoxicillin is not advised. The pathogens may contain a natural cephalosporinase, so cephalosporins such as ceftriaxone or ceftazidime are not a good choice. The pathogen is resistant to aztreonam. Since the morbidity is largely toxin-mediated, there is a possibility that systemic administration of steroids may be beneficial but good data is lacking. Pleural fluid should be drained early and aggressively since it is associated with improved survival by reducing the toxin level and by decreasing mechanical lung compression.

In 2009, a single dose of **raxibacumab** (ABthrax), a human monoclonal IgG1 antibody directed against protective antigen, the binding part of the tripartite anthrax toxin, was shown to improve survival in two animal models of inhalational anthrax, where rabbits and monkeys were exposed to approximately 200 times the lethal dose of inhalational anthrax spores. This monoclonal antibody binds protective antigen with high affinity and blocks binding of the toxin to its receptor. Safety studies of IV raxibacumab 40 mg/kg in healthy volunteers showed a half-life of about 3 weeks. It had a good safety profile. **Obiltoxaximab** is a monoclonal antibody against the protective antigen of B. anthracis. It is effective in animal models. **Hyperimmune serum** from vaccinated volunteers is beneficial in animal studies and seems promising in human infection: nineteen patients with anthrax were treated with anthrax immunoglobulin and antimicrobial therapy under an expanded access program. Three had inhalation anthrax, one had gastrointestinal anthrax, and 15 had injection anthrax caused by contaminated heroin. Of these patients, 13 survived, including two of the three patients with inhalation anthrax.

#### Cutaneous anthrax is treated for 7-10 days with fluoroquinolones or doxycycline,

although wound cultures are often already negative after 24 hours. This rule of thumb applies to people who have contracted the infection for example by handling an infected animal.

### Prevention

Cutaneous anthrax because of bioterrorism (where there is a possibility of aerogenic exposure) is treated for fully 60 days. If spores may have been inhaled, antibiotics should be



used prophylactically for a period of two months. Ciprofloxacin 500 mg bid is a good first choice and doxycycline 200 mg/day is an alternative. These antibiotics do not kill the spores but the vegetative forms. It should be emphasised that the decision to administer preventive antibiotics is determined by the probability of exposure, and not by the laboratory results of the potentially infected person. In patients requiring post-exposure prophylaxis, vaccinations spread over 3 dose (0,2 and 4 weeks) can be considered. The carcasses of infected animals should be burnt, not buried. Vaccination for animals can be carried out with an acapsular, low-virulence strain (Sterne vaccine). There is a vaccine for humans, and it is used for example for vaccinating soldiers (USA). Six injections are needed, spread over more than a year. It is given at weeks 0, 2 and 4 with subsequent injections at 6, 12 and 18 months. Thereafter, annual boosters are needed. The effectiveness of this vaccine has been demonstrated by aerosol exposure of monkeys, where full protection was established after 8 weeks falling to 88% protection after 100 weeks.

## **Biowarfare and bioterrorism**

Anthrax can be used as a **weapon for biowarfare and bioterrorism**.

#### **Use of Antrax in Wars**

In the First World War an attempt was made in Scandinavia to infect horses and reindeer with sugar lumps containing anthrax spores. The animals were used for transporting the allies' supplies. Baron Otto Karl von Rosen was arrested in 1917, suspected of sabotage and spying for Germany. It was only 80 years later that it was discovered that the sugar lumps in his bag contained anthrax. So many years after the incident, the bacterial spores were still alive.

In World War II the American forces prepared thousands of small hay balls impregnated with anthrax spores. These were shipped to England with the intention of dropping them over cattle-breeding areas of the Axis countries in order to disrupt meat supplies in Germany. The weapon was never used.

Before the first Gulf War, Iraq made large quantities of anthrax. Here too the weapon was



not used.

The toxicogenic bacteria can be cultured in vitro. To obtain a weapon that can be used in aerosol form, the formation of spores from the cultures must be promoted. The mass that is obtained is then freeze-dried and ground to a fine powder. Weapons-grade powder would be characterized by high spore concentration, uniform small particle size, particles with a certain electrostatic charge to promote mutual repulsion and an agent to prevent clumping. The spores display a tendency to stick together so that quite **large particles** are formed. Large particles do not stay airborne for a long time. Because the greatest danger comes from spores between 1 and 5  $\mu$ m, which can reach the alveoli quickly, the spore powder has to be treated in order to prevent its forming larger particles.

After they have been dispersed or whirled up, the pathogens can reach the pulmonary alveoli by inhalation, without being exhaled again immediately or being removed by mucociliary clearing. The inoculation dose for inhalational anthrax for a person is estimated at 10,000 (2,500-55,000) spores. This is quite high and explains why formerly "woolsorters' disease", even among furriers who used goat's wool was rare.

Anthrax is not spread from person to person and medical personnel do not need to use additional protective equipment apart from the usual standard hygiene precautions, an important difference compared with plague pneumonia.

#### **Bioterrorism**

In 1993, members of the Japanese Aum Shinrikyo sect repeatedly spread anthrax in Kameido, Tokyo. There were however no cases of disease, because the sect had used a non-virulent strain (vaccine strain without capsule), low spore concentration, ineffective dispersal, a clogged spray device and probably also because of inactivation by sunlight (on a bright summer day, B. anthracis spores have an estimated survival time of less than 150 minutes).

The fear that anthrax would be used in bioterrorism became reality after the attacks of September 11, 2001 on the World Trade Center, New York and the Pentagon, Washington



DC, USA. A week after the turmoil of September 11, letters containing anthrax spores were mailed to various people, government departments and news agencies in the USA. Twenty-two people developed anthrax infections, including people working in mail-sorting centres. Eleven people developed inhalation anthrax, and five of those victims died. The powder in the envelopes contained high concentrations of finely dispersed anthrax spores, made of different grades in different envelopes.

What to do if such a scenario would be repeated? The government must be informed of any incident where release of anthrax is suspected. Samples are taken for bacteriological examination from the area in which the spores are released. Afterwards, decontamination is carried out with a strong hypochlorite solution. For the people involved, who may still be asymptomatic, nasal swabs are taken and potential victims are advised to immediately wash thoroughly with soap in a shower and then to take ciprofloxacin 500 mg bid (adults) until the full result of the laboratory examination is known. The accuracy of a nasal swab culture in predicting exposure is not known, and its value is greatly disputed. There is really no good method for determining whether someone has or has not been exposed to an aerosol that contains B. anthracis. If the infection is confirmed and there are still no symptoms, ciprofloxacin PO is taken for two months. Vaccination can be considered but has never been used in these cases. The vaccine is not routinely available.

#### **Biological weapons and Rebirth Island**

Vozrozhdeniye Island, or "Rebirth Island" in English was located in the Aral Sea, which divides the Central Asian countries of Uzbekistan and Kazakhstan. (The recent drying out of the Aral Sea makes the place no longer an island). During the Soviet era, the island was an open-air testing site for the Soviet biological weapons program. From 1936 to 1991, field tests carried out on the island involved the release of "weaponized" pathogens: microorganisms specially developed by military scientists to be virulent, hardy, and antibiotic-resistant. Among the biological warfare agents tested on the island were special strains of *Bacillus anthracis* (the causative agent of anthrax), *Yersinia pestis* (plague), and *Francisella tularensis* (tularemia) that had been rendered resistant to multiple antibiotics and environmental stresses. It is likely that viral agents, including the smallpox virus, were also tested on the island.



The Red Army's Fifteenth Directorate which ran the test site, operated a year-round command post in Aralsk, on the Kazakh mainland. All of the key facilities on the island, however were located south of the Uzbek border. At the barracks and headquarters area, up to 800 scientists and troops were deployed at the peak testing periods from April to August.

The Aral Sea was once the world's fourth largest inland body of water. During the Soviet testing program, deadly germs released experimentally were unable to escape from the island because a large expanse of open water separated it from the mainland. Beginning in the 1960s the Soviet authorities diverted the sea's feeder-rivers into concrete irrigation canals, with the aim of growing large amounts of cotton. After a few successful harvests, the desert soil became exhausted, the rivers silted over, and desiccation and pesticide contamination turned the area into an environmental wasteland, with serious health consequences for the local populations. The diversion of the rivers has also caused the Aral Sea to shrink dramatically and ended the former isolation of Vozrozhdeniye Island. By the late 1980's the sea's level had dropped so much that the lake had separated into two distinct bodies: the Small Aral (north) and the Large Aral (south). By 2007 the south had split into a deep Western basis, a shallow eastern basis and a small isolated gulf. The Large Aral's volume had dropped from 708 to 75 cubic kilometers, accompanied by a rise in salinity. In 2001 Vozrozhdeniya united with the shore in the South. By 2008 the initial small landbridge become a broad base, transforming the island into a peninsula connected to the Uzbek mainland. The implications of rodents carrying infected fleas leaving this former testing ground can only be guessed at present.

In 1988, after the Soviet BW program was supposedly shut down. Large quantities of anthrax spores had been produced at the military microbiology facility in Sverdlovsk and then stockpiled near Irkutsk. Because the volume of the anthrax material was too large to autoclave, it was shipped to Vozrozhdeniye Island for decontamination and burial. The anthrax spores were mixed with bleach in 250-liter stainless steel containers and then buried in 11 pits within a total area of less than a football field. Because the spores tended to clump together, some were protected from the bleach and remained viable in the soil.

In 1992, Kanatjan Alibekov, a senior Soviet bioweapons scientist, defected to the United States and revealed that weaponized anthrax had been buried on Vozrozhdeniye Island.



The U.S. intelligence community was able to determine the locations of the burial sites from historical satellite images taken while the pits were being dug. A Department of Defence team then travelled to the island and took soil samples, which revealed the presence of viable spores of weaponized anthrax.

In the aftermath of the September 11 attacks, the U.S. government recognized the urgency of decontaminating the anthrax burial sites to eliminate the threat of terrorist access. Moreover, because oil companies are interested in drilling on the island for petroleum and natural gas, these activities could stir up contaminated dust that could blow across to the mainland. The special decontamination solution was used to soak the anthrax-contaminated soil in situ. The soil was dug up and passed through the solution again to make sure that all the spores were killed. The anthrax pits decontamination ended in late 2002.

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# Tularemia

#### Summary

- Tularemia: bacterial infection by Francisella tularensis
- Contact with infected animals (e.g. wild rabbits), contaminated dust and water
- Fever, skin lesions and lymphadenopathy
- Other presentations include ocular, septicaemic and pneumonic forms
- Diagnosis: clinical presentation, culture and/or antibodies

## General

Tularemia (syn. tularaemia) is an infectious disease caused by a small, pleomorphic, aerobic, non-motile and non-spore-forming Gram-negative coccobacillus, *Francisella tularensis* (formerly *Pasteurella tularensis*). The generic name refers to Edward Francis, a scientist who devoted many years of his life to studying the disease. The species name refers to Tulare County in California, an area where tularemia occurs regularly. There are three biovars, *F. tularensis tularensis* (biovar A, syn. nearctica), *F. tularensis holarctica* (biovar B, syn. palearctica) and *F. tularensis novicida* (biovar C).

In humans, infection with type A has a much more serious course than with type B. Type A is mainly found in rabbits and rodents. Type B is found more in animals that live near water and is predominant in Eurasia. Type A is predominant in North America, although it is sometimes found in Central Europe. Biovar C is a germ with low virulence, found in North America. **Infections in Europe or Russia tend to have a much milder course than infections in the New World.** Type A is fatal to guinea pigs and rabbits, unlike type B. Serologically there is no difference between the three forms. Both phagocytosing cells and non-phagocytosing cells can be invaded. **Intracellular multiplication** occurs. Specific exotoxins such as in anthrax have so far not been demonstrated. There is however an **endotoxin**, similar to other Gram-negative pathogens. The disease has been studied for possible use as a **biological weapon**.

# Transmission

The infection is restricted to certain areas and **only occurs in the Northern Hemisphere**: Mexico, USA, Canada, Scandinavia, eastern Europe and in Russia as far as Siberia. Cases which occurred in Utah led to the name "Pahvant Valley fever". There are few infections in Japan, where the disease is known as "yatobyoo". In 1939 some 2300 cases were reported in the USA, but since then the number of infections has fallen substantially. In 1966-67 there was an epidemic with more than 600 cases in Sweden. In the period 1999-2000, 327 cases were reported in post-war Kosovo. In the New World, cottontail rabbits and jackrabbits form an important reservoir, hence the common name "**rabbit fever**". Other animals such as dogs and cats, sheep, squirrels, skunks, beavers, muskrats and even birds can be infected. Prairie dogs can become chronic carriers. **Various occupations** are at an increased risk of tularemia: hunters, butchers, veterinary surgeons, and furriers. There have been no reports of person-to-person transmission. Transmission is by **inhalation, ingestion, inoculation or contamination through direct contact** with infected material, including water. Although the pathogen does not form spores -unlike anthrax- the bacterium can **survive for 2-6 months in mud, water and carcasses**.



Transmission can be by the bite of hard **ticks**, **fleas** or **horseflies** such as tabanids ("deer fly fever"). These arthropods first infect themselves by sucking the blood of an infected animal. With ticks there is transovarian transmission. The pathogen is present in small numbers in tick saliva and in greater numbers in tick faeces. The ticks that are notorious for transmitting *Francisella tularensis* in the USA are *Dermacentor andersoni* (Rocky Mountain wood tick), *D. variabilis* (American dog tick), *D. occidentalis* (Pacific coast dog tick) and *Amblyomma americanum* (Lone Star tick). **Skin contact** with the infected tissue of an animal that has for example been hunted and skinned is dangerous. The disease can occur after **eating infected** meat. Racoons, snakes or coyotes can carry the bacteria in their mouths.

Domestic animals or wild animals that have had direct contact with an infected animal can cause infection in man. Transmission by **aerosol** is possible. Transmission can occur by breathing in contaminated **dust** that has been whipped up, such as by a grass cutter or brush cutter. By this route the pathogen is extremely infectious. This was one of the reasons why tularemia was studied as a bio warfare agent. Fewer than 50 bacteria are enough to cause pulmonary infection. The infectious dose by the oral route is much higher: 108 organisms.

# **Clinical aspects**

The disease occurs in different clinical forms. Its presentation depends on the route of infection, the size of the inoculum, the virulence of the organism and the immune status of the patient.

**Ulceroglandular form**. About 80-90% of cases are of this form. The point of entry may be the site where an arthropod has bitten. Microtraumata with small tissue defects in the skin form a point of entry. After an incubation period of 2-4 days (1-10, exceptionally 21) there is **suddenly high fever with rigors**, together with headache, nausea, vomiting and pronounced malaise and fatigue. A primary red, slightly itching and slightly painful skin **papule** is observed. This soon becomes pustular and necrotic. The ulcer is usually on the hands. Afterwards there is **local lymphadenopathy** (buboes) with swelling of the epitrochlear and/or axillar lymph nodes. If inoculation occurs on a leg, there are swollen inguinal/femoral lymph nodes. Oral infection results in cervical lymphadenopathy. The lymph nodes may **suppurate** and drain to the skin. A non-specific roseola-like maculopapular rash



appears in 20% of cases. Rarely there is erythema nodosum.

**Oculoglandular form** (1%). With inoculation in the conjunctiva, for example due to dirty fingers, severe **painful conjunctivitis** develops, followed by swelling of the ipsilateral lymph nodes. Keratitis and corneal ulceration may follow. If the pre-auricular nodes are swollen, this is called **Parinaud's oculoglandular complex**. This is to be distinguished from cat-scratch disease, tuberculosis, sporotrichosis, sarcoidosis and syphilis. [P.S. Do not confuse the term with Parinaud's syndrome, a neurological entity with vertical gaze abnormalities due to lesions in the dorsal part of the midbrain, the colliculi superior.]

A **purely glandular** form can occur, but this is rare (2%). It is a form consisting of local lymphadenitis without a primary skin lesion. Sometimes there is cervical adenopathy, which suggests oral ingestion of the pathogens.

**Oropharyngeal form**, with stomatitis and/or severe inflammation of the throat (pharyngitis, tonsillitis) that can resemble diphtheria, together with cervical lymphadenopathy.

**Gastrointestinal form** follows eating infected meat. Mesenterial lymphadenopathy, abdominal pain, nausea, vomiting, diarrhoea and intestinal blood loss from intestinal ulcers occur.

**Typhoidal form**. Here sepsis with abdominal pain predominates. Myalgia and joint pain may occur but are unspecific. **Disseminated necrotic foci** are found throughout the body (1 mm to 8 cm in diameter). The **systemic toxicity** is pronounced. Delirium can occur. Splenomegaly and perisplenitis can arise. A full blood count reveals a normal or raised leukocyte count. Mediastinitis, meningitis, peritonitis and lung abscess can occur as complications but are rare. Tularemia is a rare cause of "fever of unknown origin".

**Pulmonary tularemia**. Tularemia is a rare cause of **atypical pneumonia as well as fulminant pneumonia**. Primary pulmonary tularemia progresses rapidly with fever, cough, dyspnoea and a burning feeling under the sternum. Pleural effusions and pleuritic pain can occur. On a chest X-ray there are poorly defined infiltrates and the concave lining of pleural fluid can be seen. Mediastinal lymphadenopathy can occur. Pneumonia does not always have to be primary but can be secondary (cfr similar situation in plague).



#### **Differential diagnosis:**

Depending on the clinical presentation, several other diseases can also be considered. The clinical picture of a **febrile syndrome of sudden onset with a skin lesion and swollen lymph nodes after contact with a possibly infected animal**, could be:

- ulceroglandular tularemia (Francisella tularensis), but also
- bubonic plague (Yersinia pestis) or
- cutaneous anthrax (Bacillus anthracis).
- Skin infection with pyogenic bacteria such as *Streptococcus pyogenes* and *Staphylococcus aureus* are in most cases not difficult to diagnose.
- Rat bite fever, also known as "sodoku" is caused by *Spirillum minus* and can follow a bite from an infected rat. Relapsing fever, skin lesions and joint pain are important.
- Dog bites are often infected with *Capnocytophaga canimorsus*.
- Scrub typhus (*Orientia tsutsugamushi*) occurs in Asia (geographically different area from tularemia). Here the lymphadenopathy is less pronounced.
- Swimming pool granuloma caused by *Mycobacterium marinum* may be a possibility, but its course is less rapid, and the general condition is excellent.
- Cat-scratch fever (Bartonella henselae) is a more difficult differential diagnosis.
- Sporotrichosis can mimic tularemia.

**Oropharyngeal tularemia** must be distinguished from diphtheria, severe streptococcal pharyngitis, actinomycosis, lymphoma, tuberculosis and Plaut-Vincent pharyngitis.

**Atypical pneumonia due to tularemia** can resemble infections caused by *Coxiella burnetii*, *Legionella pneumophila*, *Chlamydia psittaci*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and even *Histoplasma capsulatum*. Fulminant pneumonia can resemble anthrax, pneumonic plague, SARS and pulmonary hantavirosis caused by the Sin Nombre virus.

**Typhoidal tularemia** may resemble typhoid fever (Salmonella typhi), brucellosis (Brucella sp), typhus (rickettsioses such as Rocky Mountain spotted fever) and erhlichioses. The latter two should be especially considered if it is known that the person has been bitten by a tick. If granulomata are present tuberculosis and sarcoidosis can be brought into the differential diagnosis of tularemia. Haverhill fever is caused by *Actinobacillus muris* (= Streptobacillus



moniliformis) and can follow a rat bite or by drinking milk infected with rat urine. In practice the diagnosis of Haverhill fever can only be confirmed by identifying the pathogen in a culture.

# Diagnosis

*Francisella tularensis* type A is a **level 3 pathogen**. As the bacterium is highly infectious, it is dangerous to try to isolate it in a standard laboratory (culturing skin lesions, sputum, pleural fluid, blood culture). Laboratory infections have been described. It is not an easy bacterium to culture. Clinical samples can be examined quickly with fluorescing antibodies.

**Serology** is important. In some patients antibodies are positive after one week but in other patients it takes three weeks before antibodies can be detected. This can lead to false-negative results early in the disease. In the right context a single raised value of 1/160 can suggest the diagnosis. There is a limited cross-reactivity with *Brucella* and *Legionella* bacteria. These antibodies play a minor role in protection. It is predominantly primary (polymorphonuclear) and cellular immunity which is responsible for protection. The T-lymphocyte-dependent protection develops over the course of 2-4 weeks. Initially a lesion contains many neutrophils.

A biopsy of a cutaneous lesion may be pathologically similar to tuberculosis, but the evolution of tularemia is far more rapid. There is **granuloma** formation with epitheloid cells, lymphocytes and polynuclear giant cells. PCR exists for the bacterium.

## Treatment

The pathogen is sensitive to **gentamicin**, **streptomycin** and to **fluoroquinolones** and **doxycycline**. Tularemic meningitis can be managed with an aminoglycoside combined with chloramphenicol or doxycycline. If the patient is pregnant gentamicin is still the recommended treatment. If treatment is given soon after infection, mortality remains low. Skin wounds require local care. In the case of ocular tularemia moist dressings, eyedrops with homatropine and dark glasses are recommended.



## Prevention

Avoid **ticks and insect bites** (protective clothing, repellents, permethrin). Wear gloves and masks **when touching wild animals** (e.g. the fieldwork of a biologist) particularly if these are rabbits in an endemic area. Shot game must be very thoroughly cooked before it can be eaten. The previously used vaccine prepared from the live vaccine strain (LVS) of F. tularensis subspecies holarctica is no longer available because of concerns about its unknown mechanisms of attenuation and stability. Using leaf blowers to clear gardens, streets or parks in areas with tularemia is not advised (airborne transmission via contaminated dust).

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# Plague

#### Summary

- Plague: infection with Yersina pestis, a Gram-negative bacterium
- Isolated cases or epidemic
- Transmission via fleas (importance of rat population), body lice (hygiene) or aerogenically (cough)
- Lymphadenitis (bubonic plague), pneumonia (pneumonic plague) with septicaemia and bleeding
- Isolation of cases, flea and lice eradication
- Aminoglycoside (gentamicin), fluoroquinolone or tetracycline
- Tetracycline for immediate contacts

# General

Plague is an infection caused by a **Gram-negative bacterium**: **Yersinia pestis**. This organism was isolated in 1894 by the Japanese researcher Shibasaburo Kitasato (a co-worker of Koch) and the Swiss bacteriologist Alexander Yersin (a student of Pasteur) during an epidemic in Hong Kong. The organism has a characteristic shape when stained with Giemsa or Wayson stain: a bipolar rod with a safety pin appearance. The organism is non-motile and



forms no spores. The organism grows well on various tissue media. In 1897, the Japanese doctor Masaki Ogata reported that plague was transmitted by rat fleas. In 1898, Paul-Louis Simond during his work in Bombay suspected that the rat flea *Xenopsylla cheopis* might be the vector. This was confirmed experimentally in 1914 by Bacot and Martin.

#### Yersinia

Do not confuse Yersina pestis with Yersinia enterocolitica or Yersinia pseudotuberculosis. These bacteria can provoke enteritis and mesenterial adenitis (swollen lymph nodes in the mesentery, especially near the terminal ileum and the ileocolic junction). Y. pseudotuberculosis is maybe the cause of Izumi fever (pseudoscarlatina).

#### **Historical perspective**

There have been various well-known pandemics in history. The **Athenian "plague"** (430 BC) at the time of the Peloponnesian War (431-404 BC) was described by the Greek historian Thucydides, but the precise aetiology of this epidemic is uncertain. The profusion of different hypotheses (Ebola, *Rickettsia prowazekii*, ergotism, epidemic recurrent fever, smallpox, *Bacillus anthracis*, *Yersinia pestis*, arbovirosis, robovirosis, a variant of "Spanish" flu, etc.) shows that, in the absence of essential data, a correct diagnosis after the event is not easy.

In 542 AD, at the time of the Roman emperor Justinian, an epidemic occurred in Pelusium, in Egypt, a seaport at the mouth of the eastern branch of the Nile delta. The epidemic subsequently struck Turkey and Europe (**Justinian plague**). The consequences and terrors were described by the Byzantine historian Procopius, secretary to Belisarius, one of the most important generals under Emperor Justinian. The epidemic ended about 767.

In 1346 there were **cases of plague in Astrakhan**, situated at the mouth of the Volga (north of the Caspian Sea). Afterwards, spread occurred via the River Don to the Sea of Azov and subsequently to the shores of the Black Sea. In 1347 there were **Genoese traders in the city of Caffa** (now Feodosiya), in the south of the Crimean peninsula in the Black Sea. It was the terminus of the northern branch of the Trans-Asiatic silk route.



The city was besieged by Janiberg, leader of the Kipchak Tartars, in whose camp an epidemic of plague broke out. The Tartars catapulted bodies of their own comrades who died of the disease over the walls of the city. To what extent this contributed to the spread of plague is open to question. Anyway, the plague appeared in Caffa city. Twelve Genoese ships withdrew with cases of plague on board. Their crews went ashore at various places in Constantinople, Cyprus, Messina (Sicily), Southern France and Italy, after which a major epidemic broke out in December 1347. In June 1348 the plague reached Paris. In December it arrived in England. In May 1349 a ship with a cargo of wool sailed from London to Bergen in Norway. A few days later it was found drifting with the crew dead off the Norwegian coast. The cargo was brought on land and by the end of 1349 the plague had spread throughout the whole of the country. In 1351 the plague came to Poland. The Black Death in the 14th century wiped out approximately a quarter of the population of Western Europe. Together with the other terrors of the 14th century (e.g. the Hundred Years' War between England and France, 1339-1453), this meant that the **European population declined from 73 million to 45 million**.

The term "**quarantine**" stems from 1370, when seafarers arriving in the Republic of Ragusa in Southern Italy were isolated for 40 days (quaranti giorni).

**Plague also raged from the 15th to the 17th century in Europe**. The Great Plague of London in 1665 totalled 70,000 deaths. The epidemic was possibly stopped by the Great Fire of London in 1666, but according to English demographic data ("Bill of Mortality") mortality had already declined before the Great Fire.

**Subsequently other smaller outbreaks happened** (Marseilles in 1720, Egypt in 1834). The decline of the plague has been associated with the reduction in the number of black rats and their replacement by brown rats which have less close contact with humans.

**In 1860, a new epidemic arose in Yunnan, China**, which later spread, first to the town of Pakhoi and then to Canton (Guangzhou), before subsequently travelling downstream and reaching Hong Kong in 1894. It was then that the organism was isolated. From this port there was further spread via ships' rats (e.g. to San Francisco 1903, Auckland, Bangkok, Manila, Rangoon, Saigon, Batavia, Tokyo, Sydney, Cape Town, Buenos Aires, Mauritius and Glasgow), which caused huge mortality, especially in India. Between 1898



and 1918, 8 to 12.5 million people died in India. The epidemic was brought to a halt in the first half of the twentieth century. In North China there was also a major epidemic. This resulted from the intensified hunting of marmots. These mammals had a valuable pelt and were also very susceptible to plague. The local Mongols knew the risk of this only too well and shot the animals instead of catching them. They also always avoided touching sick or dead animals. When the price of pelts quadrupled in 1910, there was a large influx of inexperienced amateur Chinese who hunted without precautions in search of rapid profits. The hunters also often kept warm together in underground shelters, which was ideal for transmission. Pneumonic plague broke out in Hailar and spread along the railway line to Harbin and afterwards to Vladivostok.

In the Second World War, Japanese Imperial Army's Unit 731 killed thousands of Chinese and Russians held prisoner in Japanese-occupied Manchuria, in experiments to develop chemical and **biological weapons**. Japanese doctors tested the use of plague among others. Infected *Pulex irritans* fleas were cultured and released in a few Chinese towns, resulting in small epidemics of bubonic plague.

After an absence of 50 years, plague reappeared in 2003 in **Oran and in other foci in Algeria**. New foci were discovered in 2008, including one in Libya. The rodent species *Meriones shawii* (Shaw's jird) was shown to be present in the transmission area. The animal is plague-resistant and forms an efficient reservoir for *Yersina pestis*.





Spread of plague throughout Europe during Middle-Ages

#### **Plague = plague?**

How do we know so positively that the "plague" in earlier centuries was in fact "the plague"? Naturally, there are numerous historical descriptions that are suggestive, but there still remains questions. In the case of the Athenian plague there are many question marks regarding the aetiology. There have also sometimes been epidemics of diseases with high mortality which disappeared as quickly as they had appeared and which do not resemble any disease that we now recognise (e.g. the epidemic of lethal "sweating sickness" (1485-1551) which in the summers of 1508, 1517, 1528 and 1551, claimed many victims in England and elsewhere). The nature of the organism that caused "sweating sickness" is still unknown. In 1998, Didier Raoult (Marseilles) studied the dental pulp of non-erupted teeth from people who had died in the 16th and 18th century from



plague and were buried in large graves in Lambesc and Marseilles. Using PCR technology it was possible to detect a few genes of *Yersinia pestis* in the dentition. Control teeth were negative. This technique opens new avenues for study and for obtaining a better understanding of historical epidemics.

### **Present situation**

Plague is at present a rare, cosmopolitan disease which still persists in **various foci in several parts of the world**. From 2000 to 2009, a total of 21,725 cases of plague with 1612 deaths (7.4 percent fatality rate) were reported worldwide from 16 countries. A further 3248 cases of plague were reported to the World Health Organization (WHO) between 2010 and 2015, with 584 associated deaths. Since 2000, more than 95 percent of reported cases have been from Africa. Outbreaks of human plague, with numbers of cases ranging from 100 to more than 1000, have occurred since 1992 in DRC, Peru, India, and the Congo. Plague reappeared in Malawi, Mozambique, and India in 1994, in Algeria in 2003, and in Libya in 2009, raising concern that the disease may re-emerge as a worldwide public health hazard. Available data may be underestimates because diagnostic facilities and surveillance systems are inadequate in many areas of the world where plague is endemic or occurs in focal outbreaks.

In the Western World, the rate of plague is low, probably because the affected areas are rural and largely uninhabited. In the United States, a total of 91 cases of human plague were reported in the United States from 2000 to 2015, over 80 percent of which were the bubonic form.





Map showing areas where plague occurred in the period 1970-1998

## **Transmission and epidemiology**

Plague is first and foremost a disease of **wild rodents** (**zoonosis**). Mammals from at least 73 genera can be infected and approximately 30 species of fleas can transmit the organism. This does not mean that they are all equally important. Many of these animals are relatively resistant to the infection. Only a few are of importance for maintaining enzootic and epizootic cycles. In a focus of infection, it is possible to obtain an idea of the local situation (plague surveillance) by serological surveys of various wild animals. Sometimes an epizootic occurs (an epidemic in animals).

### World Distribution of Plague, 1998



#### **Paul-Louis Simond**

French researcher Paul-Louis Simond (1858-1947) helped in Bombay to combat the Indian plague epidemic of 1897. At that time, it was thought that rats caught plague by cannibalising dead rats, and that people caught plague through tiny cuts and cracks in their feet. Simond showed it was rather difficult to infect rats by feeding them infected material. Also, mere physical contact with infectious material did not seem to infect the rats. However, pricking the feet of rats with a plague-contaminated needle infected them rather easily. Rubbing plague material on the surface of an intact rat paw produced no infection. If rats could get plague via tiny prick injuries, what might be causing them in their natural habitat? Simond considered insect bites. He knew rats were often infested with fleas. He also knew rat fleas would bite humans (fleas are less discriminatory of food sources than lice). In a critical experiment, he showed that rats did not get plague in the absence of fleas. Simond noted that not only were there large number of dead and dying rats in the streets and buildings, but that 20 laborers in a wool factory who had been cleaning the floor of dead rats had died of plague, but none of the other factory workers who had no contact with rats had become ill. He found that healthy rats groomed themselves and had few fleas, while sick rats unable to groom their fur had many. When the rats died, the fleas moved on to other hosts. Simond began to suspect fleas as intermediaries. In an experiment, he placed a sick rat at the bottom of a jar and suspended a healthy rat in a wire mesh cage above it. Although the healthy rat had no direct contact with the plague-infected one, it did become infected. Simond determined that rat fleas could jump 10 cm high without difficulties. As a control he placed a sick rat without fleas together with healthy rats in a jar. None of the healthy rats became sick (which ruled out air-borne transmission). When he introduced fleas into the jar, they developed plague and died. On 2 June 1898 he wrote Pasteur that the problem of plague transmission had been solved. It would be several years before he was believed.

The bacteria can survive for a long time in the burrows of various rodents. The infection is transmitted **from animal to animal by fleas**. When a flea sucks blood from an infected animal it ingests bacteria. These organisms then proliferate in the insect's proventriculus and stomach. The bacteria attach to the wall if they carry a specific gene, the "haemin storage locus". At the same time, they secrete an enzyme (coagulase) that coagulates the aspirated blood. This causes an **obstruction in the flea's stomach**. The flea then becomes



increasingly hungry and bites more often. As a result of the obstruction, the **blood with bacteria is regurgitated**. The flea can only digest the clots at temperatures higher than 28°C ("cold fleas digest poorly"). At high environmental temperatures (>28°C) a plague epidemic will therefore spread less rapidly and sometimes stop because the flea can digest the blood and there is much less regurgitation into the bite wound. The proventriculus of the flea in fact contains internal projections which make regurgitation difficult in "usual" circumstances. The bacteria can also be introduced into a wound by flea faeces or by crushing the insect (scratching an itchy fleabite!).



Cat flea. Ctenocephalides felis. Occasional vector of Yersinia pestis (plague) and vector of Rickettsia felis. Copyright ITM



An isolated case of plague can occur when a human is bitten by an infected flea from wild rodents such as sand rats or desert rats [gerbils] (e.g. *Meriones* sp, *Tatera* sp, *Rhombomys* sp, *Gerbillus* sp). This is then referred to as **sylvatic transmission** ("sylva" = wood). This happens for instance to hunters, wood cutters, etc. Other animals, such as *Mastomys* sp, *Arvicanthis*, *Otomys* sp, etc., are also involved in transmission but are less important. Carnivores of the cat and dog families and species belonging to the weasel family naturally have a high probability of **being contaminated by their prey** as a result of their hunting behaviour. There are regular cases of transmission via a sick domestic cat or dog. **These animals can cough and infect humans aerogenically**. Contamination can also occur through wounds and direct contact with contaminated body fluids. Consumption of contaminated meat and liver (e.g. sick camel) can result in active infection with *Y. pestis*.

Sometimes **rodents that live close to humans are infected**. Rats, principally the brown rat (*Rattus norvegicus*, also called the Norwegian, grey or sewer rat; little contact with humans) and the black rat (*Rattus rattus*, also known as the house rat, lives close to humans) constitute the main reservoir. These rats are much more susceptible to infection than gerbils. The **plague bacterium usually kills the rat**, after which the flea *Xenopsylla cheopis* – the oriental rat flea – has to search for another source of blood, often humans. There are other fleas (e.g. *Pulex irritans* [human flea], *Nosopsyllus fasciatus* [brown rat flea], *Oropsylla montana* [rock squirrel flea], *Oropsylla silantievi* [tarabagan flea]) that can transmit plague, but these are of minor epidemiological importance. It is possible that transmission via *Pulex* was very important during the period of the Black Death in Europe.

*Y. pestis* may have a **reservoir in the soil**. It has been shown that *Y. pestis* can survive for at least 24 days in contaminated soil under natural condition. The upper limit is unknown at present.

The presence of *Y. pestis* in the fleas affects their behaviour, such as their preferred optimal temperature. Infected fleas appear to prefer a mean environmental temperature that is  $1.6^{\circ}$ C lower than that of non-infected fleas. Healthy rats have a body temperature of  $\pm$  38.5°C. Sick rats develop fever (i.e. >38.5°C). Thus, **infected fleas are unlikely to remain on an infected rat.** They move on to the next available host. If this is a human, then the bacterium is transferred at the same time. This has important consequences in the epidemiology of the infection with the massive release of contaminated fleas in the event of extensive rodent die-



off ("ratfall"). **Humans are then accidental "hosts" to the fleas**. In this case, human-tohuman transmission still does not occur.

**Epidemic plague** can occur e.g. via bites from the human flea ("Pulex irritans"). A patient with bubonic plague can develop **secondary pneumonic plague**. When humans develop the pulmonary form of plague, the disease **can be further transmitted from person to person by cough droplets** without further intervention by fleas or rats.

In the USA, there are several cases of plague every year following contact with sick or dead wild animals (mice, squirrels, prairie dogs, rabbits, etc). *Oropsylla montana* is an important vector in the USA. Monitoring rodent populations and their predators (e.g. coyotes) is important for predicting imminent outbreaks. It should be noted that domestic cats, dogs and other animals can also be infected with plague and develop the disease.

Historical data seem to imply that rat-die offs were not associated with human epidemics in the 1300's. The rodent's fleas might not have been active during the cold European winter months. Still cases of bubonic plague occurred (besides pneumonic plague) during the cold periods, very suggestive of transmission via biting arthropods. It was demonstrated that **body lice** can also transmit plague. Since they stay in human clothing, transmission during winter can be expected. Body lice can be infected when living on a septicaemic patient and stay alive for a week, producing infectious faeces. The exact role of body lice is still not well defined, but further work might clarify the epidemiology of this disease.

# Yersinia pestis

Three biotypes of the bacterium are currently recognised based on the capability of glycerol fermention and nitrite to nitrate conversion. Ribotyping of the various isolates supports the recognised division of these biotypes. These are the Antiqua, Mediaevalis and Orientalis biotypes. The Antiqua biotype occurs in Africa, Southern Russia and Central Asia. The Mediaevalis biotype is found around the Caspian Sea. The Orientalis biotype is predominant in Asia and is the only one that occurs in the New World. A fourth biotype, Microtus, refers to Medievalis isolates lacking arabinose fermentation. In 1951, Devignat proposed that each of the first 3 biotypes determined each plague pandemic.



However, at present there are strong arguments to suppose that the three historic pandemics were caused by the Orientalis biotype (studies based on PCR-analysis of ancient dental pulp of victims).

## **Clinical aspects**

Some cases are asymptomatic. After a flea bite, a local pustule or ulcer occurs, sometimes with a black crust. The bacterium spreads via the lymphatics. Some cases have clinical features of minor lymphadenitis.

#### **Bubonic plague**

The incubation period is short (2-7 days). In a minority of cases (6%), there is a pustule or a carbuncle at the site of the flea bite. In most cases, no ascending lymphangitis is noted. Sudden high **fever with chills** occurs, associated with hypotension, headache and severe general malaise. The **regional lymph nodes** draining the site of the bite enlarge rapidly and are very painful. In most cases, the femoral and inguinal lymph nodes are affected, followed in terms of frequency by the axillary and cervical nodes. Plague nodes differ from other lymphadenitides through their rapid development, severe pain and accompanying toxaemia. Mild forms however also occur ("pestis minor"). The swollen lymph nodes are known as **buboes**, from which the term "bubonic plague" is derived. The buboes rapidly break open, discharging dirty, foul-smelling, necrotic tissue. There is high fever and the patient's general condition is poor, blood pressure low and the liver and spleen can be enlarged. Subcapsular splenic bleeding is not unusual. Mortality is high (50-90%). With rapid treatment it can be reduced to 1-2%. Blood vessels are damaged and contain clots. Subcutaneous bleeding occurs, which takes the form of petechiae, purpura and ecchymoses. Subsequently, the skin lesions become necrotic and gangrene can set in ("Black Death"). If treatment is incomplete, meningeal invasion can occur (plague meningitis). When pustules or ecthyma gangrenosum are the predominant clinical features, this is sometimes referred to as cutaneous plague.

#### Septicaemic plague

Sometimes **sepsis/septic shock** is clinically apparent before the lymph nodes have time to enlarge: septicaemic plague. This is an incorrect term since septicaemia also occurs in the



other forms of plague. **Bacteraemia** can be very high so that sometimes bacilli can be seen in a thin or thick blood smear. Often the patient presents initially with gastro-intestinal symptoms, such as nausea, vomiting, diarrhoea and/or abdominal pain, which can lead a clinician astray. In most cases the patient dies very rapidly (1 to 2 days) in a condition of septic shock with refractory hypotension, renal failure, stupor, ARDS and DIC (petechiae, bruising, bleeding tendency and acral gangrene).

#### **Pneumonic plague**

These days, pneumonic plague is rare. The infection can be **primary** as a result of contamination via an aerosol of plague bacteria or **secondary** through haematogenic spread to the lungs. Primary pneumonic plague has an incubation period of 2 to 4 days. The onset is acute, and the course is fulminant with fever, chest discomfort, general malaise, hypotension and severe pneumonia, with a productive cough and bloody sputum. This is usually associated with pleural effusion. Patients who cough are very contagious. At this point another person can be infected by direct person-to-person transmission. It takes the form of a **very rapidly progressive pneumonia** with almost 100% mortality within a few days. Secondary pneumonic plague initially takes the form of interstitial pneumonia with a small amount of thick, viscous sputum, subsequently progressing to the symptoms described above. It is striking how unremarkable the auscultatory findings are. It is possible but not formally proven, that *Yersinia pestis* increases its virulence after repeated passage via the lungs.

#### **Oropharyngeal plague**

Oropharyngeal plague, in which the portal of entry is the throat (ingested flea, consumption of contaminated meat, dirty hands after touching contaminated animal tissues), takes the form of a serious disease with throat pain, severely enlarged painful cervical lymph nodes and local oedema (DD diphtheria, anthrax, tularemia).

### Diagnosis

Consideration should be given to the possibility of plague, particularly if there is a sudden increase in **rodent mortality in an endemic region**. The diagnosis should be considered in



healthy subjects who suddenly become very severely ill with fever, extremely enlarged painful lymph nodes, brutal pneumonia or if a rapid succession of deaths occurs within one family.

Extensive **leukocytosis** is present. **Microscopic examination** of aspirated fluid from a bubo, sputum, cerebrospinal fluid and/or peripheral blood shows bipolar Gram-negative bacilli. The buboes do not contain liquid pus. Some sterile saline (1 ml) is injected into a bubo in order to obtain an aspirate. In the words of Yersin, the fluid contains "une véritable purée de microbes". Sometimes the bacteria can be detected in a thick or thin **blood smear**. They then have the appearance of a "safety pin" (bipolar granules). A staining method that reveals this clearly is the Wayson stain (based on basic fuchsin mixed with methylene blue in 95% ethanol and phenol). The organism is then light blue with darker terminal granules.

**Culture** is desirable for formal proof in view of the implications of a potentially threatening epidemic.

Serology is possible in specialised laboratories (e.g. ELISA for detecting antibodies to the F1 antigen). Approximately 5% of survivors do not seroconvert. Serology permits a retrospective diagnosis, but is not useful for the acute, individual patient.

There is also a technique available involving a dipstick coated with antibodies which can be used to detect **the F1 antigen**. This rapid test can use sputum or serum, as early as the second day of the disease. The result is known in 15 minutes and is thus clinically very useful for the individual patient and any contacts. F1-deficient mutants occur very rarely and cannot be detected with this dipstick method.

Presumptive identification of Y. pestis can also be made by polymerase chain reaction (PCR). PCR testing has been used to detect Y. pestis in skeletons which are hundreds of years old.





Blood smear with Yersina pestis bacteria. Copyright ITM

#### **Differential diagnosis:**

**Bubonic** plague, with its principal characteristic feature of acute buboes, need to be distinguished from:

- lymphogranuloma venereum (much slower progression)
- chancroid (slower, ulcers, fluctuating bubo)
- streptococcal/staphylococcal adenitis (general condition is good)
- filarial adenitis (progression, microfilaria, eosinophils)
- strangulated inguinal hernia.



**Pneumonic** plague takes the form of a rapidly progressing pneumonia. It can resemble

- a brutal bacterial pneumonia (e.g. pneumococcal)
- legionellosis, tularaemia
- anthrax, SARS (Coronaviral pneumonia)
- or hantavirus pulmonary syndrome (Sin Nombre virus).

An isolated case can be easily missed. In epidemics, there is the possibility that all pulmonary symptoms of all patients are attributed to pneumonic plague (e.g. patients with pneumococcal pneumonia may be viewed as having pneumonic plague).

**Septicaemic** plague develops very rapidly and resembles meningococcal septicaemia or other severe forms of Gram-negative sepsis. Confusion with acute rickettsioses (epidemic typhus) and louse-borne relapsing fever is possible.

# Therapy

All patients should be **isolated**, including those with bubonic plague, because secondary pneumonic plague can develop. In 1948 it was discovered that **streptomycin** was active against the plague bacillus and this antibiotic still remains the first choice. In view of the high mortality and rapid progression, treatment must be initiated as soon as possible. The dose of streptomycin for adults is 2 x 1.5 g IM daily. If streptomycin is not available, gentamicin constitutes a good alternative. For gentamicin, a dose of 2 mg/kg tid is used. Hypotension should be treated, preferably with IV fluids. Improvement is rapid and most patients are afebrile after 3 days. It is not necessary to combine antibiotics. It is important to maintain therapy for at least 10 days.

**Tetracyclines** are an alternative to aminoglycosides: 2 to 4 g orally for 10 days. They are also very useful in epidemics. Quinolones are also active however not as effective and often are more expensive.

Chloramphenicol is indicated in plague meningitis and/or endophthalmitis. Initially it is given IV. After a few days, in most cases it becomes possible to switch to oral medication. Sulphonamides are also used as prophylaxis, but they are not the first choice. **Penicillins,**  **cephalosporins and macrolides are inactive** against *Yersinia pestis*. Resistance to the common antibiotics is infrequent. Sometimes tetracycline-resistant strains are isolated. In 1995, a **multiresistant strain of** *Yersinia pestis* was isolated in Madagascar (resistance to streptomycin, kanamycin, chloramphenicol, tetracyclines, sulphonamides, ampicillin and spectinomycin). The resistance was coded by a plasmid. *Yersinia pestis* probably acquired the plasmid via horizontal transfer from another Gram-negative organism of the *Enterobacteriaceae* family.

## Surveillance

Surveillance can be conducted in several ways. Carnivores can be regularly tested serologically and constitute a sensitive sentinel system of rodent plague in a specific area. *Yersinia pestis* can be detected in animals found dead in a region. The fleas can be collected from abandoned rodent nests, identified and tested. Live rodents can be captured and these animals and their fleas examined.

# Prevention

Plague is a disease for which **international quarantine is mandatory** and cases must be **notified**. All patients with plague, irrespective of the presence of cough or pneumonia, should be treated in **strict isolation for at least 48 hours** (risk of secondary pneumonic plague with subsequent aerogenic transmission). The room should be decontaminated and sprayed with insecticides. Masks, goggles and protective clothing are indicated. Gloves should be worn when handling bubonic aspirates and blood.

Contacts may take tetracyclines (4 x 500 mg) or vibramycin for 1 week (ciprofloxacine or sulphonamides are an alternative). They should be closely monitored for 7-10 days.

Vaccination gives temporary protection against bubonic plague, but the vaccine is very difficult to obtain. Soldiers in the American forces during the Vietnam War were routinely vaccinated with a dead cell vaccine (3 primary injections followed by boosters, depending on the antibody titre in the blood). There was a much lower incidence in vaccinated than in the South Vietnamese forces (1/3000 cases per year of exposure).



**Urban plague** can usually be controlled by **quarantine** and by **rat control and flea eradication**. **Sylvatic plague cannot definitively be eradicated** in view of its animal reservoir. In combating urban plague, **fleas should be controlled first and then the rats**. Otherwise a large number of fleas are suddenly released (since they no longer have any animal host) and then transfer to humans. It is important to have an idea of the susceptibility of the insects to various insecticides. As strains of *Xenopsylla cheopsis* and *Synosyllus fonquerniei* (flea vectors in Madagascar) have been found which were resistant to the insecticides DDT and dieldrin (organochlorine compounds), malathion or phenitrothion (organophosphates) and propoxur (carbamate). Such **resistance** data are useful if there is an outbreak.

Rat control involves the use of various methods, including rodenticides such as anticoagulants (warfarin, fumarin, bromadiolone, chlorophacinone), zinc phosphide, sodium fluoroacetate and strychnine. Rats are very social and intelligent animals and can learn to avoid poison, as well as teaching their nest mates to do so.

The concern about plague as a bioterrorism agent has led to the development of several newer vaccines, some of which are undergoing clinical testing.

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# **Brucellosis**

### Summary

- Gram-negative coccobacilli (*Brucella* spp.) with a tropism for the reticulo-endothelial system
- Zoonosis, through infected dairy products and animal contact (goats, sheep, cattle)
- Chronic granulomatous infectious disease
- Chronic fever and wide range of symptoms
- Diagnosis by serology and culture
- Treatment by rifampicin, doxycycline, aminoglycoside for at least 6 weeks



# General

Brucellosis is a **chronic granulomatous infectious disease** caused by small, facultative intracellular, Gram-negative coccobacilli. *Brucella melitensis* (goats, sheep, camels, chamois, ibex), *B. abortus* (cattle, buffalo, bison, zebra, impala, waterbuck, hippopotamus), *B. suis* (pigs) and *B. canis* (dogs) are the causative agents of this **zoonosis**, in descending order of importance. There are several biovars. For example; pigs are infected by *B. suis* biovars 1, 2 and 3, European wild rabbits by biovar 2. Biovar 4 is found in caribou and reindeer. Humans are accidentally infected and play no role in the survival of these organisms in nature. Animals are the only source of infection and **there are no known vectors**. *B. ovis* (sheep) and *B. neotomae* (desert rats) are not known to cause disease in man. Other species (*Brucella pinnipediae, B. maris, B. cetaceae*) infect marine mammals, such as seals, dolphins, porpoises, minke whales, etc. There have been rare cases of human infection with some of these marine strains.

#### Historical

The condition was known as **Malta fever** as a result of a persistent epidemic at the end of the 19th-century in British soldiers on the island. The disease was studied intensively by David Bruce of *Trypanosoma* fame. He studied 91 cases and found two features: **splenomegaly and undulating fever**. In 1887 he isolated the organism from splenic tissue of dead soldiers and named it "*Micrococcus melitensis*". This organism was capable of infecting healthy chimpanzees. In 1897, Wright described a serum agglutination test for the diagnosis of this disease. In 1904 the Brucella Committee was established, as a result of which it was possible to undertake large-scale epidemiological research. In 1905, Themistocles Zammit discovered that the blood of many, apparently healthy goats agglutinated *Brucella* organisms. Bruce identified the organism in goat blood and milk and as such discovered the reservoir of the organism. Up to 10% of animals had *Brucella* in their milk. Monkeys which received infected goat's milk to drink developed the disease.

After some hesitation, specific measures were implemented. **Pasteurization** was introduced as a legal requirement in Malta in 1938. The transport of goats was restricted, infected goats had to be killed and milk had to be boiled or pasteurised, including the milk



used for the preparation of cheese. The ban on using fresh milk resulted in a dramatic fall in the number of cases in the British Army, but the reduction of cases in the island population was much less spectacular because the indigenous population did not accept the idea of boiling milk. The last documented outbreak of brucellosis on the island occurred in 1995.

In 1895-1897 the Danish doctor/veterinarian Bernhard Bang (1848-1932) identified *Brucella abortus* in cows, the pathogen of infectious abortion in these animals. A previous name for brucellosis was "Bang's disease". In 1921, a substantial problem of brucellosis was seen in Rhodesia in people who had had no contact with goats. However, there was often infectious abortions seen in livestock. Apparently *Brucella abortus* could also infect humans. So, there appeared to be more than one organism that caused undulating fever.

In 1914 Traum identified *B. suis* in pigs. Carmichael and Bruner discovered *B. canis* in 1968 in dogs. *B. pinnipediae* and *B. cetaceae* were only discovered in 1994 by Ewalt and Ross.

## **Transmission**

Transmission of brucellosis occurs mainly through **eating or drinking contaminated unpasteurized animal-milk products** such as raw milk, soft cheese (cottage cheese), butter and ice cream. Hard cheese, yogurt and sour milk are less dangerous because of the fermentation which has taken place. Eating undercooked infected animal products (spleen, liver) are occasionally responsible for infection. A low pH in the stomach is partially protective (importance of antacids, ranitidine, omeprazole, etc.). **Direct contact** (inoculation through skin wound, conjunctiva) with secretions and excretions of infected animals (e.g. placenta, aborted foetuses) can also cause disease. Pregnant infected animals usually develop placentitis. Inhalation of **infected aerosolized particles** can occur (personnel working in microbiology labs!). This has been studied in the context of biowarfare. Brucellosis is an **occupational disease** in farmers, livestock producers, herdsmen, butchers, veterinarians, shepherds, abattoir workers, dairy-industry professionals and lab workers. There is almost no human-to-human transmission although in rare cases sexual transmission has been suspected. The organism has been isolated from human breast milk and from sperm. In



animals the disease is commonly transmitted sexually.

After entering the human body and being taken up by local tissue lymphocytes the bacteria migrate via the regional lymph nodes into the general circulation. They display a tropism for the **reticuloendothelial system**. *Brucella* bacteria **replicate intracellularly** without affecting cellular viability. They switch off cellular apoptosis rendering the host cell immortal.

## **Clinical aspects**

The clinical features are **very varied and often non-specific**. The incubation period is usually **two to four weeks** but can be as short as one week or as long as several months. The temperature is often only raised in the evening. General malaise, various symptoms such as sweating, headache, muscle pain, abdominal pain, tiredness, depression, etc., may occur. Sometimes the clinical presentation is that of **fever of unknown origin**. Chronic febrile arthritis should point to brucellosis (and tuberculosis). Some patients try to explain their joint or bone lesions as being due to local trauma, whereas the real cause is a *Brucella* infection. Osteomyelitis of the vertebrae can resemble tuberculosis (Pott's disease). Sacroiliitis, arthritis of the sternoclavicular joints and involvement of the large joints (hip, knee) is not unusual. The fever can occur in waves ("**undulant fever**"). Uveitis, both posterior and anterior, can be found. Brucellosis can mimic various other diseases and is one of the great "imitators" in the world of infectious diseases. Rarely peripheral neuritis, orchitis, meningitis, cholecystitis, aortitis or endocarditis can be seen as a consequence. Neurobrucellosis is a feared complication. The risk of abortion in women is thought to be much lower than in animals.

On physical examination, **splenomegaly is observed in 25%** sometimes with enlarged lymph nodes in the groin and neck. Skin abnormalities (papules, erythema nodosum, fine erythematous rash) can occur, but is found only in a minority of cases (5%). There can be signs of arthritis in general large joints (hip, knee, or the sarcoiliac joints). The clinical findings in neurobrucellosis depend on the localisation of the lesions. A slitlamp eye examination and ophthalmoscopy should always be included in any physical examination.

Physical examination usually does not provide pathognomonic findings. Above all the **possibility of brucellosis should be considered** in the differential diagnosis. With the cluster of orchitis arthralgia-eye problems, consideration should first be given to Reiter's



syndrome rather than to brucellosis, although brucellosis can lead to such symptoms.

# Diagnosis

**Leukopenia** or a normal white blood cell count is more common than leukocytosis. Normocytic **anaemia** is frequently present. Sometimes there is thrombocytopenia. Liver tests may be abnormal and a liver biopsy or bone marrow specimen can often (± 75%) show **granulomatous lesions**. If granuloma are large enough, they can display fibrinoid necrosis. The cerebrospinal fluid can be abnormal with an **increased lymphocyte count**, raised CSF protein and normal glucose concentration.

Brucellosis can be suspected **serologically**, but the antibodies cross-react with, for example, *Yersinia enterocolitica, Francisella tularensis, Salmonella* and other organisms. Serologically, *B. canis* infections can be detected only with difficulty. False negative results are common early in the course of infection. A prozone effect can also occur (negative serology at low dilutions becoming positive at higher dilutions). There are rare cases of active Brucella infections in which the standard serology is negative ("blocking antibodies"?). Many laboratories use the so-called "**Rose Bengal**" test, an agglutination test which gives results within 5 minutes. If positive, a **Wright** serological test can be performed but this test needs a longer time (serum agglutination test with overnight incubation). After successful therapy, the IgG titre falls.

**Isolation of the organism** from blood, tissue, urine, bone marrow, cerebrospinal fluid, require specific culture media. It is a **slow-growing organism**. It is best to notify the laboratory beforehand. Bone marrow cultures have a higher sensitivity than blood cultures. With some rapid automated commercial methods, misidentification of the organism as *Moraxella phenylpyruvica* is possible. Because the organism is a coccobacillus, a laboratory can wrongly describe the organism as a coccus on one occasion and as a rod-shaped bacterium on another.

Radiographs, bone scans, computerized tomography (CT), magnetic resonance imaging (MRI), and echocardiography may be helpful in evaluating focal disease but do not provide a definitive diagnosis. Localized snowflake calcification in chronic hepatosplenic brucellosis is the only specific radiographic finding that may be used to distinguish brucellosis from other



diseases. PCR is a promising tool for rapid and accurate diagnosis of human brucellosis.

## Treatment

**Rifampicin** (600-900 mg/day) and **doxycycline** (200 mg/day) are often used as first line. If possible an **aminoglycoside** should be added (minimal dual regimen; optimal tritherapy which includes streptomycin or gentamycin). Sometimes combination treatment includes cotrimoxazole (children, pregnant women) or ofloxacin. It is recommended that a specialist with experience in brucellosis be consulted. Treatment lasts **at least six weeks**, but sometimes must be continued for many months. In general, longer courses of therapy (at least 12 weeks) are warranted for treatment of spondylitis, neurobrucellosis, endocarditis or localized suppurative lesions. Clinical relapse sometimes occurs, usually within 6 months of discontinuing the antibiotics. Relapse is usually not a consequence of antibiotic resistance, but due to the persistence of a focus (drainage sometimes necessary). Naturally, patients can still complain of pain following correct treatment due to the consequences of joint involvement, for example.

It was found (with real-time PCR) that in the majority, *Brucella melitensis* **DNA will persist** in the human body for several years despite appropriate treatment and apparent clinical recovery. It has not been formally shown that this DNA is from dead or living bacteria, but it strongly suggests that *B. melitensis* is a noneradicable persisting pathogen.

## **Prevention**

**Detection and destruction of infected animals** must be implemented. Brucellosis may be prevented via **vaccination**, which is effective for cattle, sheep and goats (not for humans), but requires a sustained vaccination program over several years. **Proper pasteurisation of milk** and avoidance of cheese made from potentially contaminated milk are important. If for example cottage cheese is used in cooking, it needs to be heated long enough (the centre heats less quickly than the outside; the centre of the lumps needs to be heated above the minimum temperature to destroy *Brucella* bacteria). Gloves are to be used when working with potentially infected animals and their secretions.



#### **Uveitis**

Uveitis is a general term for inflammatory disorders of the uveal tract. Anterior uveitis is the term which encompasses iritis and iridocyclitis. Posterior uveitis is the preferred term for choroiditis and chorioretinitis. In the non-granulomatous form, the onset is characteristically acute, with pain, injection, photophobia and blurred vision. There is a circumcorneal flush caused by dilated limbal blood vessels. Fine white deposits on the posterior surface of the cornea can be seen with a slitlamp. The pupil is small and there may be a collection of fibrin with cells in the anterior chamber. If posterior synechiae are present, the pupil will be irregular in shape. In granulomatous uveitis, the onset is usually insidious. Vision gradually becomes blurred and the affected eye becomes diffusely red with circumcorneal flush. Pain is minimal and photophobia is less marked than in the nongranulomatous form. Fresh active lesions of the choroid and retina appear as yellowishwhite patches seen hazily with the ophthalmoscope through the cloudy vitreous. As healing progresses, the vitreous haze lessens and pigmentation occurs gradually at the edges of the yellowish-white spots. In the healed stage there is usually considerable pigment deposition. If the macula is not involved, recovery of central vision is complete. The patient is usually not aware of the scotoma in the peripheral field corresponding to the scarred area.

There are various causes, including several infectious diseases, but also auto-immune disorders. A wider range of diagnoses must be considered for patients in developing countries. Expert advice from an experienced ophthalmologist and a specialist in internal diseases is essential to save the patient's sight. If for example toxocariosis of the eye were to be treated with anthelminthics only, the larva would die and release a large quantity of antigen. This would cause the intra-ocular inflammation to increase, resulting in cloudiness of the vitreous humour and total blindness.

#### Infectious causes of uveitis include

1.Parasitic: toxoplasmosis, Toxocara infection (infection by the larva of a canine nematode), cysticercosis (larval Taenia solium), Onchocerca volvulus microfilaria



2.Bacterial: syphilis, tuberculosis (with granulomata on the retina), leprosy, bartonellosis with cat scratch disease, leptospirosis, Q fever, Lyme disease, brucellosis

3.Viral: CMV (think of HIV), herpes simplex, HTLV-1, measles

4.Fungal: Candida (usually panophthalmitis), cryptococcosis, histoplasmosis

#### Non-infectious causes include:

- 1.Sarcoidosis
- 2.Systemic lupus erythematosus (i.e vasculitis).

3.Traumatic and sympathetic ophthalmia.

4.Reiter's syndrome. In addition to anterior uveitis, conjunctivitis, urethritis, balanitis, oral ulcers, low fever and joint pain can also be present. There is often a recent history of infected sexual contact (Chlamydia trachomatis) or enteritis. Hyperkeratotic lesions on the palms of the hands and soles of the feet resembling pustular psoriasis can occur.

5.Associated with juvenile rheumatoid arthritis, Still's disease.

6.Associated with ankylosing spondylitis – HLA B27 (Bechterew's disease).

7.Behçet's syndrome.

8.Vogt-Koyanagi-Harada syndrome (uveo-encephalitis) with cutaneous and neurological symptoms in addition to ocular lesions (birdshot retinopathy).

9. Unknown cause, e.g. heterochromic uveitis (Fuch's cyclitis)

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# Melioidosis

#### Summary

- Environmental bacterium (soil, water): Burkholderia pseudomallei
- Southeast Asia and Northern Australia are hotspots
- Infection is through skin and inhalation
- Diabetes and other immune depressed at risk
- Acute or chronic disease
- Skin infection pneumonia blood stream infection deep abscesses, high mortality
- Treatment: ceftazidime or meropenem followed by co-trimoxazole or co-amoxiclav (at least 3 months)

## General

Burkholderia pseudomallei (formerly Pseudomonas pseudomallei) is a facultative intracellular Gram-negative rod-shaped bacterium also known as Whitmore's bacillus. The organism is responsible for infections in sheep, goats, pigs, cattle, horses, rats, cats and dogs. **Soil and stagnant water (rice fields)** form its natural reservoir. Humans are infected by **contaminated soil via skin** abrasions. **Swallowing and inhalation** of the bacilli can also result in clinical infection. Neonates can be infected on rare occasions (via placental microabscesses?). The disease is endemic in **Southeast Asia and northern Australia**. Very rarely cases are diagnosed in Central and South America and also in Africa.

*B. pseudomallei* has two chromosomes. Together they contain more than 7 megabasepairs, making it a very complex bacterial genome. Genotyping of multiple *B. pseudomallei* colonies from several tissue sites showed substantial genetic diversity within a single patient, illustrating the capacity of the bacterium to evolve rapidly within a host. It can invade and survive in a range of phagocytic and non-phagocytic cells. It replicates in the cytosol after leaving the vacuole.



#### **Historical perspective**

Glanders is a chronic disease of horses associated with involvement of the nasal mucosa with mucus production, as well as local lymph node enlargement. Glanders in animals is caused by the immotile *Burkholderia mallei* (formerly *Pseudomonas mallei*). Human infections are rare.

In 1911, the British pathologist Captain Alfred Whitmore and his assistant C.S. Krishnaswami discovered that ill-nourished and neglected inhabitants of Rangoon, Burma, exhibited the same sort of lesions as horses with glanders. They also performed autopsies on emaciated morphine addicts. About one in every twenty post-mortem examinations in Rangoon Central Hospital was on a case of the disease. The organism which was recovered from the numerous and widespread abscesses observed at post-mortem examination in these cases could be grown on peptone agar or on potato slopes (the bacteriological tools of the day). The organism isolated from humans, however exhibited some differences from the one that caused glanders in animals. The new bacterium was motile (glanders is caused by an immotile bacterium) and caused a slightly different reaction after inoculation in guinea pigs. The bacterium was initially called Bacillus *pseudomallei*. The term "pseudoglanders" is sometimes used in English. In 1913 there was an outbreak of an unusual "distemper-like" disease in the veterinary department of the Institute for Medical Research in Kuala Lumpur, Federated Malay States. Dr Fletcher isolated the organism during this outbreak, but he was unable to identify it. In 1917 Stanton isolated the bacterium during an outbreak among Tamil rubber tappers, and saw it was identical to Whitmore's bacillus. In the following years Stanton and Fletcher conducted research on this organism and named the disease meioidosis (Gr. "melis", referring to glanders-like disease of asses).

The occurrence of infections in Vietnam in French colonial soldiers involved in a car accident led to the hypothesis that the organism could enter the body via mud-soiled wounds or via aspiration of muddy water. Guinea pigs with a scarified abdomen could be infected by immersion in muddy water. Finally, the organism was cultured in vitro from soil. It was shown that the organism produced a heat-labile exotoxin. During the Vietnam War several cases occurred in wounded soldiers, but there were also abnormally large



numbers of cases among helicopter pilots, which suggested that aerogenic transmission was possible. Several American veterans developed active melioidosis up to 26 years after their stay in Vietnam. An 82-year-old U.S. veteran held as a Japanese prisoner of war in Indochina during World War II developed an infected ulcer on his right hand as symptom of melioidosis. This was 62 years after his exposure. No-one knows the anatomical site where the bacterium survives or how the immune system is evaded. All in all, our knowledge about melioidosis is clearly inadequate. There is a strong association between **melioidosis and rainfall** (80% of cases occur in the wet season). Heavy rain and wind, such as in monsoon season seems to cause a shift from inoculation towards inhalation of *Burkholderia pseudomallei*.

In 1950 there was an epidemic in Aruba – an island off the coast of Venezuela. In 1970 an outbreak in France was linked to the zoo in the Jardin des Plantes near the Musée National d'Histoire Naturelle. It was assumed that the epidemic was caused either by an infected giant panda imported from China or an infected horse introduced from Iran.

### **Clinical aspects**

The incubation period can last **weeks, months or years**. Subclinical infections can occur. The disease can be latent for years. Often the clinical presentation is that of an acute febrile respiratory infection (**pneumonia**), **but acute localized skin infection** (skin abscess with or without drainage sinus, necrotizing fasciitis, lymphangitis), **blood stream infection** with or without a clear focus, genitourinary infection, synovitis with or without septic arthritis, osteomyelitis, neurological involvement (myelitis, brain-stem encephalitis with cranial-nerve palsies) and chronic disease with **disseminated organ abscesses** also occur. Suppurative parotitis seems to be common in Thailand and Cambodia but is very rare elsewhere. **Pure cutaneous** forms without systemic features exist, from a primary solitary lesion to multiple lesions (secondary spread). Pustular rash can be found during septicaemia. Respiratory tract infection is sometimes difficult to distinguish from tuberculosis (both classical and miliary). Pulmonary cavities can appear. Splenomegaly is regularly present. During pulmonary melioidosis, urticaria, flushing and/or cyanosis can occur. In some areas, such as northern Thailand, it is the most important cause of community-acquired bloodstream infection.



Meloidosis is one of the "great imitators" due to its wide -ranging clinical presentation

Melioidosis tends to have a **protracted course** and cure is difficult without a **prolonged course** of appropriate antibiotics.

**Risk factors** include alcoholism, malnutrition, renal failure, chronic pulmonary disease, corticosteroid use, cancer and especially diabetes. There is insufficient data about a possible interactions with HIV. Mortality in active disseminated disease is high, about **40-80%**, especially when additional risk factors are present. With early diagnosis and institution of therapy with ceftazidime or meropenem and access to state-of-the-art intensive care therapy, the overall mortality from melioidosis can now be as low as 10 percent.

# Diagnosis

Patients tend to be from Southeast Asia (esp Northeast Thailand, Cambodia) or Northern Australia. The infection can be suspected from a chest X-ray. The diagnosis is established by **culture** (blood, urine, skin, sputum). The organism grows on several media but should be distinguished from *Pseudomonas* species. Growth can be quite slow, as compared with other bacteria that cause blood stream infection. In view of the risk which this organism presents, culture and isolation is best left to well-equipped laboratories. Gram stain of sputum and abscess pus may reveal gram-negative bacilli of B. pseudomallei. The organisms often have a characteristic bipolar staining with a "safety pin" appearance.

Antibodies can be detected serologically. A positive serology can point to an active infection or a previous (including subclinical) melioidosis. Most seropositive patients have no overt clinical disease. A latex agglutination test which can be used with urine has been developed. The main differential diagnoses are tuberculosis, disseminated fungal infections and chronic pyogenic osteomyelitis but melioidosis is one of the "great imitators". It is clear that more research is needed.

# Treatment

*Burkholderia pseudomallei* is **intrinsically resistant to numerous antibiotics**, including aminoglycosides, penicillin, ampicillin, first- and second-generation cephalosporins,



chloramphenicol and fluoroquinolones. First line treatment for severe cases is IV **ceftazidime** (Glazidim®, a beta-lactam belonging to the third generation cephalosporins) combined with cotrimoxazole. Dosage is ceftazidime 2 grams TDS for a minimum of 2 weeks. Beta-lactams belonging to the carbapenems such as imipenem in combination with cilastatin (Tienam®) or meropenem (Meronem®) are (expensive and often difficult-to-access) alternatives.

For mild ambulatory cases, amoxicillin with clavulanic acid (co-**amoxiclav**, Augmentin), also in combination with high dose **cotrimoxazole** forte 3 to 4 tablets per day for an adult (one tablet containing trimethoprim 160 mg + sulfamethoxazole 800 mg) is often used.

The optimal duration of maintenance treatment (cotrimoxazole or doxycycline) is not known but **3-6 months** is often recommended.

**Relapse** can occur after several years, especially during immunosuppression. This means that lifelong follow-up is indicated.

There is currently no vaccine available.

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