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.


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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 µm, 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.