Bacteria
Melioidosis .................................................................................................................. 123
  General ................................................................................................................. 123
  Clinical aspects ................................................................................................. 125
  Diagnosis ............................................................................................................ 126
  Treatment ............................................................................................................ 126
Rickettsiosis and related infections ........................................................................... 127
Rickettsiosis .............................................................................................................. 127
  Classifications .................................................................................................... 129
  Transmission ....................................................................................................... 130
  Clinical aspects ................................................................................................... 137
  Diagnosis ............................................................................................................. 141
  Treatment ............................................................................................................ 143
  Prevention ............................................................................................................ 143
Q-Fever ...................................................................................................................... 144
  General ................................................................................................................ 144
  Epidemiology ..................................................................................................... 145
  Clinical aspects ................................................................................................... 146
  Diagnosis ............................................................................................................. 147
  Treatment ............................................................................................................ 148
  Prevention ............................................................................................................ 149
Ehrlichia and Anaplasma ........................................................................................ 150
  Historical note ................................................................................................... 150
  Transmission and infection ............................................................................... 150
  Diagnosis ............................................................................................................. 151
  Treatment ............................................................................................................ 154
Carrion’s disease ..................................................................................................... 154
  History ................................................................................................................ 154
  Aetiology .............................................................................................................. 157
  Distribution ........................................................................................................ 158
  Transmission ....................................................................................................... 158
  Clinical aspects ................................................................................................... 158
Carrion’s disease ..................................................................................................... 159
  History ................................................................................................................ 159
  Aetiology .............................................................................................................. 163
  Distribution ........................................................................................................ 164
  Transmission ....................................................................................................... 164
  Clinical aspects ................................................................................................... 164
Verruga peruviana .................................................................................................. 165
  Immunity ............................................................................................................. 168
  Diagnosis ............................................................................................................. 168
  Treatment ............................................................................................................ 169
  Disease control .................................................................................................. 170
<table>
<thead>
<tr>
<th>Disease</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bartonella quintana</strong></td>
<td>170</td>
</tr>
<tr>
<td>General</td>
<td>170</td>
</tr>
<tr>
<td>Transmission</td>
<td>170</td>
</tr>
<tr>
<td>Clinical aspects</td>
<td>171</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>173</td>
</tr>
<tr>
<td>Treatment</td>
<td>173</td>
</tr>
<tr>
<td><strong>Cat-scratch disease</strong></td>
<td>173</td>
</tr>
<tr>
<td><strong>Spirochaetal diseases</strong></td>
<td>174</td>
</tr>
<tr>
<td><strong>Non-venereal treponematoses</strong></td>
<td>175</td>
</tr>
<tr>
<td>Bejel or Njovera or Treponarid</td>
<td>176</td>
</tr>
<tr>
<td>Framboesia or Yaws or Pian</td>
<td>177</td>
</tr>
<tr>
<td>Pinta</td>
<td>190</td>
</tr>
<tr>
<td>Summary</td>
<td>192</td>
</tr>
<tr>
<td><strong>Leptospirosis</strong></td>
<td>192</td>
</tr>
<tr>
<td>General</td>
<td>193</td>
</tr>
<tr>
<td>Taxonomy</td>
<td>193</td>
</tr>
<tr>
<td>Transmission</td>
<td>194</td>
</tr>
<tr>
<td>Clinical aspects</td>
<td>194</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>197</td>
</tr>
<tr>
<td>Treatment</td>
<td>198</td>
</tr>
<tr>
<td>Prevention</td>
<td>199</td>
</tr>
<tr>
<td><strong>Borreliosis</strong></td>
<td>199</td>
</tr>
<tr>
<td><strong>Relapsing fever</strong></td>
<td>199</td>
</tr>
<tr>
<td>General</td>
<td>200</td>
</tr>
<tr>
<td>Epidemic, louse-borne relapsing fever</td>
<td>200</td>
</tr>
<tr>
<td>Endemic, tick-borne relapsing fever</td>
<td>201</td>
</tr>
<tr>
<td>Clinical Aspects</td>
<td>202</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>203</td>
</tr>
<tr>
<td>Treatment</td>
<td>203</td>
</tr>
<tr>
<td>Prevention</td>
<td>203</td>
</tr>
<tr>
<td><strong>Borrelia vincenti</strong></td>
<td>204</td>
</tr>
<tr>
<td><strong>Rat Bite Fever</strong></td>
<td>204</td>
</tr>
<tr>
<td>General</td>
<td>205</td>
</tr>
<tr>
<td>Clinical aspects</td>
<td>205</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>206</td>
</tr>
<tr>
<td>Treatment</td>
<td>207</td>
</tr>
<tr>
<td><strong>Trachoma</strong></td>
<td>207</td>
</tr>
<tr>
<td>General</td>
<td>207</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>209</td>
</tr>
<tr>
<td>Clinical aspects</td>
<td>209</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>210</td>
</tr>
<tr>
<td>Treatment</td>
<td>210</td>
</tr>
</tbody>
</table>
Typhoid fever and other salmonellosis ................................................................. 211
  General ........................................................................................................ 211
  Epidemiology .............................................................................................. 213
  Transmission ............................................................................................... 215
  Pathophysiology .......................................................................................... 215
  Clinical aspects ............................................................................................ 216
  Diagnosis ...................................................................................................... 219
  Other arguments .......................................................................................... 220
  Treatment ...................................................................................................... 221
  Prevention ..................................................................................................... 221
  Non-typhoid Salmonella blood stream infection ........................................... 222

Cholera ........................................................................................................... 222
  General ........................................................................................................ 223
  Epidemiology .............................................................................................. 225
  Transmission ............................................................................................... 228
  Physiopathology ......................................................................................... 230
  Clinical aspects ............................................................................................ 232
  Diagnosis ...................................................................................................... 234
  Treatment ...................................................................................................... 235
  Prevention ..................................................................................................... 239
  Vaccination .................................................................................................... 239

Diarrhea in the tropics .................................................................................. 242
  General ........................................................................................................ 242
  Aetiology ...................................................................................................... 243
  Acute diarrhoea ............................................................................................ 244
  Chronic diarrhoea ....................................................................................... 247
  Assessment of a patient with diarrhoea ......................................................... 250
  Treatment ...................................................................................................... 252
  Prevention ..................................................................................................... 254

Tropical sprue ................................................................................................ 255
  General ........................................................................................................ 255
  Clinical aspects ............................................................................................ 257
  Diagnosis ...................................................................................................... 257
  Treatment ...................................................................................................... 258
Bacteria

Select the topic you want to read more about.

LAST UPDATED BY ADMIN ON AUGUST 16TH, 2023

Tuberculosis

Under review

LAST UPDATED BY ADMIN ON JUNE 24TH, 2022

Leprosy

Summary

- Chronic infection with *Mycobacterium leprae*
- Bacteria multiply in the macrophages and Schwann cells of peripheral nerves
- Clinical spectrum: from tuberculoid (paucibacillary) to lepromatous (multibacillary)
- Thickened nerves with neuritis: trophic, motor and sensory disturbances
- Neuropathy leads to paralysis, trophic ulcers, blindness, mutilations
- No central nervous system lesions
- Skin: numb white area with elevated edge (tuberculoid) to diffuse infiltration with nodules (lepromatous).
- In lepromatous leprosy also involvement of deeper tissues (testes, tongue, eyes, etc.)
- Diagnosis: clinical, Ziehl staining of smears (skin lesion, nose, earlobe)
- Treatment of leprosy with dapsone, rifampicin, +/- clofazimine
- Leprosy reactions: type 1 (change in immunologic defence) and type 2 (immune complex)
- IRIS reaction possible in HIV patients within 4 months of starting HAART

General

Hansen’s disease or leprosy was previously present in most parts of the world. Now it is a problem in regions of extreme poverty. The number of registered cases is falling: 5.37 million in 1985, 3.1 million
in 1992, 1.8 million in 2000, 249,007 in 2008, and 215,656 new cases in 2013 according to WHO. At the end of 2013 the prevalence was estimated at 180 618. There are probably as many patients who have not yet been diagnosed. The number of severe infections (with disability) is clearly decreasing, reflecting earlier detection. It is hoped to bring the general incidence of the infection below 1/10,000 in the near future. A prevalence of less than 1/10,000 is regarded as the goal for eliminating leprosy as a public health problem but this is not the same as eradication of the disease. By 1999, 80% of all leprosy cases were occurring in 6 countries: India, Brazil, Bangladesh, Indonesia, Myanmar and Nigeria. HIV-infected patients usually die of infections caused by faster growing bacteria (e.g. *Mycobacterium leprae*), and not from the slow-growing *Mycobacterium leprae*. The AIDS epidemic has therefore had little effect on the incidence of leprosy but immune reconstitution after starting HAART can lead to florid lesions in a patient who had subclinical asymptomatic leprosy. The illness is characterized by skin and nerve lesions. This leads to neural dysfunction, which together with progressive tissue destruction causes mutilation. Resistance to dapsone became a significant problem around 1980. Thus combination therapy has been used since that time.

**Historical note**

Due to the mutilations which can occur in leprosy, since ancient time there has been a lot of prejudice and stigmatization. Sufferers were usually banished from the community. Apart from the physical handicap, the emotional, economic and social consequences were often very severe. The hypothesis was that leprosy was a hereditary disease and/or a punishment from God. One argument in favour of hereditary transmission or rather against the hypothesis of leprosy being an infectious disease was the result of transmission experiments, in which Dr Daniel Danielssen in Bergen, Norway, injected himself and four helpers with material obtained from skin nodules from leprosy patients, without further consequences. The pathogen of this chronic disease was discovered in 1873 by the Norwegian Gerhard Henrick Armauer Hansen. At that time there were several thousand leprosy sufferers in Norway. Following the example of John Snow (see Cholera) he followed the course of each illness over time. Families of which the members lived physically close together had a higher incidence of the disease, compared to families of which the members lived apart. In this way he came to the idea that this could be an infectious disease. In 1871-72 he observed small vague intracellular rods in skin nodules. This information was published in 1873. A staining method was discovered in 1880 by the German Albert Neisser. The pathogen proved to be a bacterium: *Mycobacterium leprae*. It was the first time that a bacterium had been considered responsible for causing a human disease. Regrettably, Dr Hansen carried out an unethical experiment, in which he introduced material from a leprosy nodule into the cornea of another person in an attempt to prove its infectious
In 1873 Jozef de Veuster, better known as Father Damian arrived on Molokai in the Hawaiian archipelago. There he found 800 leprosy patients who were living in miserable conditions. He decided to stay and to devote the rest of his life to improving the fate of his fellow human beings. In 1876 he developed lesions on his arms and back (an illustration of the long incubation period). In 1881 he developed nerve pain and in 1883 his left foot lost all sensation. He died on 15th April 1889.

**Mycobacterium leprae**

*Mycobacterium leprae* is an obligate intracellular, slow-growing acid fast bacillus (0.5 x 3-8 µm). On Gram-staining it is Gram-variable. The *Mycobacterium leprae* genome project sequenced the entire genome in 2001. The genome is rather small (3.27 Mbp) and contains about 1600 genes and more than 1100 pseudogenes. In comparison, *Mycobacterium tuberculosis* contains about 4000 genes. This seems to imply massive gene decay in the leprosy bacillus and absence of critical enzymatic pathways thereby relying on host parasitism for survival.

**Biological information**

Do not confuse *Mycobacterium leprae* with *Mycobacterium lepraemurium*, a natural pathogen of rats and mice. The disease caused by *Mycobacterium lepraemurium* is sometimes used as a model for human leprosy. In 2008, *Mycobacterium lepromatosis* was identified (analysis of 16s rRNA gene) as a related but distinct mycobacterium which might be responsible for diffuse cutaneous leprosy and Lucio’s phenomenon in humans. Additional research still has to identify the place in the overall pathology of the disease.

Phenolic glycolipid-1 (PGL-1) is a glycolipid in the capsule of *Mycobacterium leprae*. PGL-1 contains an antigenically distinct trisaccharide unit that is not found in any other bacteria. PGL-1 makes up to 2% of the total bacteria mass, suggesting that the function of the sugar chains may be related to functions unique to *Mycobacterium leprae*. PGL-1 binds to laminin-2, which facilitates PGL-1 binding to the basal lamina of axons on Schwann cells and the resulting invasion of the cells. This might explain the neurotropism of these bacteria. Because this invasion can occur even when the bacteria are dead, the invasion seems not to be driven by the bacteria, but by passive interaction between glycolipids in the capsule of the cell wall and molecules in the basal lamina of Schwann cells. If this binding can be blocked, a new therapeutic avenue may become possible. However,
laminin-2 is also present in the basement membrane of other tissues. The basement membrane in muscle is composed of laminin, type IV collagen, entactin/nidogen and heparan sulphate proteoglycan. One major component of the basement membrane in muscle is laminin-2, which is composed of a heavy chain laminin a2 and two light chains, b1 and laminin g1. Other factors must also play a role in the fact that *Mycobacterium leprae* has a predilection for neural tissue.

It has long been suspected that leprosy has a strong genetic component. A leprosy susceptibility locus on the long arm of chromosome 6 (region q25-q26) was discovered in 2003/4. This DNA stretch included the Parkinson’s disease gene *PARK2* and the co-regulated gene *PACRG*. The *PARK2* gene is expressed by human Schwann cells and macrophages, which are the primary host cells of *Mycobacterium leprae*.

**Mycobacterial culture**

It has not been possible to date to culture the bacterium in vitro, which has made research extremely difficult. This can be circumvented to some extent by making use of animal experiments. However, the bacterium multiplies very slowly (generation time 12 days). It was assumed that the bacterium had a preference for cooler parts of the body. In 1960 the American Charles Shepard (CDC) discovered that it is possible to culture the bacterium in the footpads of mice (average 30°C). In this way it was possible to obtain 10⁶ bacteria from each footpad. More severe infection could be obtained by using immune deficient mice (e.g. athymic nude mice). It became possible to test the efficacy of drugs against the mycobacterium. In 1971 Waldeman Kirchheimer and Eleanor Storrs discovered that the nine-banded armadillo, *Dasypus novemcinctus*, could also become infected. This species was selected because it has a low body temperature (approximately 34°C) and a primitive immune system. The animal develops a generalized infection with involvement of the internal organs, especially the liver and spleen. After intravenous inoculation, between 10¹⁰ and 10¹² mycobacteria per gram of tissue can be obtained (chiefly from the liver and spleen). In this way it became possible for researchers to analyse large amounts of proteins and DNA, which accelerated research. Latest data suggest that in South America armadillos might function as a natural reservoir for this infection but more study is required, clarification of this would be very important regarding the possibility of eventual eradication of the disease.

**Transmission**

Humans form the reservoir. Infection with *M. leprae* is possible in chimpanzees, Rhesus monkeys,
mangabey monkeys and wild armadillos but the epidemiological importance of this is unknown and is probably very small. Further research is required to understand its significance. In 2011 genetic analysis showed that in some cases in the Southern USA, leprosy might be acquired from infected armadillos. Leprosy in such cases can be considered as a zoonosis.

The route (or routes) of transmission is (are) at present not known with certainty. There is probable transmission via nasal secretions from humans with multibacillary leprosy. The affected nasal mucosa in these patients contains large quantities of bacilli unlike chronic skin wounds. *Mycobacterium leprae* is identified in the oral mucosa from paucibacillary and multibacillary leprosy patients. Speaking, coughing and sneezing produces aerosols (droplet clouds). The most important port of entry is probably the lungs: the bacteria are breathed in. Direct contact is probably of much lesser importance. Long-term close contact with leprosy sufferers increases the risk of infection. Nevertheless most cases occur without known contact with leprosy patients. The risk of the disease for leprosy health workers is very small. Leprosy is possibly a highly infectious disease with low disease expression. Most people exhibit no symptoms after infection whilst others have brief cutaneous lesions. The susceptible individuals are in the minority: fewer than 10% of infected people become ill. In hyperendemic regions the proportion of people with symptoms is not more than 4% and usually the ratio is even smaller (1/1000). Trans placental infection in untreated multibacillary pregnant patients has been described but is rare (in approximately 1% of the children in this situation).

**Leprosy epidemic?**

Leprosy epidemics do not occur, although a single unusual exception has been recorded. In 1912 a woman with leprosy arrived in the Oceanic island state of Nauru. This had presumably never happened before. In 1920 the first secondary case was diagnosed in the indigenous population. In 1924 there were 284 cases in a population of 1250 people. In 1929 there were 438 cases (34% of the population), after which the incidence decreased. More than 90% of the lesions were tuberculoid and deformities were rare. The extent to which genetic inbreeding within an immunologically naive population was important in this case, has not been investigated. (Compare with the ravages caused by measles in isolated island dwellers when they were first contacted by Western seafarers; see also the results of smallpox in the Aztec kingdom after the arrival of Cortez in the 16th century).

**Physiopathology**

It is assumed that after being inhaled the bacterium multiplies within macrophages and Schwann cells
(myelin-producing cells situated around peripheral nerves) and spreads very slowly. This occurs chiefly at the relatively cooler superficial body parts: the skin, superficial nerves, eyes, nasal mucosa and testicles. *Mycobacterium leprae* seems to be a thermophobic germ. Very rarely (in lepromatous patients) the bacteria spread to the deeper tissues (lymph nodes, muscles, bone, even kidneys). The central nervous system is never affected. Nystagmus, ataxia or the presence of Babinski’s sign cannot be attributed to leprosy. The human body defends itself against this mycobacterium by means of specific defence cells (lymphocytes). Tuberculoid leprosy is characterized by few bacteria and a strong Th1 immunity response. Patients with lepromatous leprosy have lesions with many bacteria and a strong Th2 immunity (with reciprocal repression of the Th1 response). If the Th1 reaction is strong, there are few bacteria and well-defined granuloma. If it is minimal, the bacteria can multiply virtually unhindered and granuloma formation diminishes. The reason why a person develops a Th1 or Th2 response to *M. leprae* is not yet clear. In lepromatous cases the high bacillary load leads to an abundance of mycobacterial antigen, which leads to immune complexes when bound to antibodies. These circulating immune complexes bind complement in order to opsonize them and facilitate uptake by phagocytes.

**Leprosy classification**

The symptoms vary greatly. This has led to considerable confusion in the past. A fundamental breakthrough was achieved by Ridley and Jopling (1962, 1964). They concluded that the clinical expression is determined by the patient’s cellular defences. They proposed a classification for the disease with tuberculoid leprosy at one extreme and lepromatous leprosy at the other and a spectrum of borderline (B) forms in between:

\[
TT \leftrightarrow BT \leftrightarrow BB \leftrightarrow BL \leftrightarrow LL
\]

In practice, this classification is complex and requires a high level of expertise and experience. Even so, consensus is difficult to reach in a single patient. Some classification schemes include polar forms (TTp and LLp). A simpler pragmatic division into paucibacillary and multibacillary forms was promoted by the WHO for operational reasons and accepted in 1987 (pauci = few; multi = many). If at least 1 acid-fast rod is found, the patient is referred to as multibacillary. This is a strategy which is still being discussed. One alternative is to regard patients who exhibit 1+ in microscopic examination as paucibacillary. One disadvantage of this very simple classification is that if the microscopy is poorly executed a multibacillary case may be classified as paucibacillary and will then remain under-treated. Another alternative classification is based on clinical grounds. Patients with 1 to 5 skin lesions and maximally 1 trunk nerve affected, are regarded as paucibacillary. If there are more than 5 skin lesions
or more than 1 trunk nerve involved, the patient is regarded as multibacillary.

**Paucibacillary:** Indeterminate, TT, BT (with no acid-fast rods on the smear)

**Multibacillary:** BT with bacteria visible on a smear, BB, BL, LL

---

**The Ridley-Jopling classification reflects the cellular resistance of the patient:**

A patient with the tuberculoid form (TT) has high cellular resistance. There are few bacteria, the lesions are localised, and the patient is not very infectious. If leprosy bacillus antigen (lepromine) is injected into the skin, the lymphocytes react strongly. A local reaction is observed. The lepromine test is positive in this case. The reaction is read after 28 days (Mitsuda reaction): diameter > 5 mm is highly positive (cf. Mantoux reaction in tuberculosis). An earlier reaction (Fernandez reaction: 48 hours) can also be read but it is non-specific. There is no cross reaction between Mantoux and the lepromine test. There is quite a poor correlation between the Fernandez and Mitsuda reactions.

There is little immunological resistance in lepromatous form (LL). There are countless bacilli and the lesions are diffuse. Patients are infectious for their environment. The lack of resistance is reflected in the negative lepromine test. The patient produces antibodies but these are not protective.

---

**Clinical aspects**

**Indeterminate leprosy**

The majority of infections do not give rise to symptoms (only to a positive lepromine test). After infection there is an incubation period of 2-15 years (the mycobacteria multiply slowly). If the patient does not recover spontaneously, a transient indeterminate lesion appears. It consists of one or more grouped hypopigmented non-pruritic macules which are well delineated. On white skin they are red or hyperpigmented. There will rarely be any reduction of sensitivity (hypo-aesthesia). Sometimes somewhat reduced sweating is seen. Nerves never become thickened at this stage. Bacilli are practically never found in this lesion. After the initial lesion there is evolution towards recovery or towards one of the forms in the spectrum TT – LL which usually occurs within 2 years. Approximately 75% of indeterminate leprosy cases recover spontaneously. This indeterminate stage is often not diagnosed. Some leprosy infections can be diagnosed on clinical grounds alone especially in family members of an untreated leprosy patient. In other situations, diagnosis is often only possible via
Tuberculoid leprosy

In tuberculoid leprosy, there are only one or a few, asymmetrical skin spots on not more than two parts of the body. They are sharply delineated, sometimes with a slightly elevated border and central healing. There are often papules on the edge. The lesion is rather hypopigmented (on dark skin) or erythematous (on white skin) and there is loss of sensitivity. First the sensitivity to temperature decreases, then the sense of touch, then pain and finally deep sensitivity. There is hair loss and the skin is dry. One or two peripheral nerves are affected (thickened), at the areas of predilection or in the region of the skin lesion. The consequences of neural dysfunction appear early (muscle weakness and atrophy, reduced sensitivity to pain, sense of touch and sweating). Paralysis is common. It sometimes occurs before the loss of sensitivity. There is no direct involvement of other tissues. Leprosy is the only infectious disease which causes thickening of the nerves. Purely neurological involvement also sometimes occurs without skin abnormalities (= neural leprosy). This slow form of neural dysfunction stands in sharp contrast to the swift neurological damage which occurs during leprosy reactions. In leprosy autonomic symptoms such as bladder or bowel problems, postural hypotension, impotence, etc are rare. Patients with amyloidosis tend to have pronounced autonomic neuropathy.

Peripheral neuropathy, mononeuritis multiplex and polyneuropathy

Most cases with leprosy present with skin and neurological signs. However, pure neuritic leprosy also occurs. In the tropics, leprosy should therefore be considered in the differential diagnosis of any peripheral neurological symptom. This tends to be predominant axonal (lower amplitudes on EMG), probably due to intraneuronal oedema with compression of the axons, but occasionally accompanying demyelination is found (slower conduction speed on EMG). Here the differential diagnosis becomes much more difficult and sometimes can only be reached on nerve biopsy.
Indeterminate leprosy, small lesion on left upper arm. The lesion was initially treated for suspected mycosis. Copyright ITM
An individual peripheral nerve can become damaged by direct trauma, invasion by a tumour, but also via entrapment e.g. carpal tunnel syndrome, repeated compression, such as prolonged leaning on an arm in a certain position (N. radialis) or repeated pressure, e.g. at the level of the fibula head while seated on the ground (N. fibularis).

The peripheral nerve damage in leprosy (outside from leprosy reactions) is due to a slow evolving mononeuritis multiplex, i.e. dysfunction of individual named peripheral nerves. In leprosy reactions, evolution is much faster, and this can lead a clinician astray especially if skin lesions are few. Sometimes the distinction with polyneuropathy with typical symmetrical distal gloves-and-stocking distribution is not so clear. The differential diagnosis of mononeuritis multiplex is vast. Many systemic diseases associated with mononeuritis multiplex cause nerve damage by affecting the vasa vasorum. Inflammation of these structures should be looked for in a biopsy when vasculitis is a possibility. Mononeuritis multiplex occurs in several forms of vasculitis (polyarteritis nodosa, Granulomatosis with Polyangitis, systemic lupus erythematosus, livedoid vasculopathy), other connective tissue diseases (mixed forms), anti-phospholipid antibody syndrome, cryoglobulinemia, sarcoidosis, amyloidosis, diabetes and as a paraneoplastic entity. Nerve lesions secondary to chronic hypereosinophilia will orient the clinician in a very different direction. Neuropathy due to diphtheria occurs about a month after infection, with mainly demyelination of motor fibers, e.g. of motoric cranial nerves, leading to visual symptoms. Lyme disease can give acute neuritis and so will usually not enter the differential diagnosis.

**Peripheral neuropathy differential diagnosis**

Lepromatous leprosy is a cause of peripheral neuropathy, leading to glove-and-stocking parasthesia. The differential diagnosis is large: many cases of polyneuropathy are secondary to metabolic disturbances and intoxications, ethanol being the prime example. Some metabolic diseases can be interpreted as autointoxication, e.g. uraemia. The clinician has to consider diabetes mellitus, hypothyroidism, vitamin 12 deficiency, dry beriberi (thiamine deficiency without cardiac failure), dysglobulinemia including multiple myeloma and Waldenström macroglobulinemia, primary and secondary amyloidosis, chronic hepatitis, heavy metal intoxication (lead, arsenic, thallium, mercury), solvents (hexacarbon solvents and CS₂), buckthorn toxin (used as tea), chronic ethylene oxide poisoning. Check for possible side-effects of medication, such as isoniazid (vitamin B6 antagonism), vincristine, cisplatinum, nitrofurantoin. Rarely
mononucleosis, typhoid fever and mumps are mentioned as causes but the pathogenesis here is unclear. Guillain-Barré syndrome is an acute ascending inflammatory demyelinating polyradiculoneuropathy and in its acute form the distinction with leprosy is straightforward. Chronic inflammatory demyelinising polyneuropathy (CIDP) however is more difficult. It resembles a chronic form of Guillain-Barré and can occur in isolation or in AIDS. A paraneoplastic origin of polyneuropathy is often difficult to prove early in the disease (lung, pancreas, ...) but as times passes, the presence and identity of the tumour will become clear. CIDP is a chronic progressive or relapsing symmetric sensorimotor disorder, leading to generalized thickening of the brachial and lumbar plexi and peripheral nerves (including sciatic nerves and others), as can be demonstrated on whole body magnetic resonance neurography. A number of hereditary conditions can lead to neuropathy, e.g. porphyria, Tangier’s disease (genetic disorder of cholesterol transport), Bassen-Kornzweig syndrome (vitamin E deficiency due to abetalipoproteinemia), Fabry’s disease (lysosomal storage disease: check family history and look for corneal opacities and spoke-like cataracts), Refsum’s disease (phytanic acid accumulation often with deafness and retinitis pigmentosa). Hereditary polyneuropathies such as the different types of Charcot-Marie-Tooth (early drop-foot, hammer toes and peroneal atrophy with thin “stork legs” with familial clustering), Déjerine-Sottas (more rapid and severe than “classic” Charcot-Marie-Tooth), Friedreich’s disease and hereditary pressure neuropathy fall need the assessment of a specialist in neurology. Finally, many polyneuropathies are idiopathic.

### Nerve thickening

Leprosy is the only infectious disease which causes nerve thickening. Nerve thickening may also occur in rare non-infectious disorders such as certain forms of primary amyloidosis of the nerves and inherited muscular and nervous diseases. Déjerine-Sottas disease is a rare form of hypertrophic neuritis which usually leads to severe disability in childhood. Here, the skin is normal. In some cases of Charcot-Marie-Tooth disease (hereditary sensimotor neuropathy type I), hypertrophic neuritis occurs. In Refsum’s disease, an autosomal recessive familial disorder, there is a defect in the degradation of phytanic acid, which sometimes causes thickening of nerves, together with cerebellar ataxia, progressive deafness, heart problems, skeletal deformations, retinitis pigmentosa and dry skin (ichthyosis). Neurofibromatosis can also be included in the differential diagnosis (including café-au-lait patches). Traumatic injury may sometimes cause local thickening, as may amyloidosis. Chronic inflammatory demyelinating neuropathy can lead to generalized diffuse thickening of plexi and peripheral nerves, as mentioned above. The technique to demonstrate this in a non-invasive way is whole-body magnetic resonance neurography using diffusion-weighted whole body imaging with background signal suppression (DWIBS) is at the onset
of the second decade of the 21st Century only available in a few medical centres in the West.

Tuberculoid leprosy, hypopigmented skin lesion. Photo Dr Brouwers. Copyright ITM
Multiple well demarcated hypopigmented skin lesions in leprosy. Photo Dr Brouwers, Copyright ITM

**Borderline leprosy**

Patients with borderline leprosy have lesions which fall between the tuberculoid and lepromatous
forms. Multiple skin lesions exist, and nerve lesions are common. Three types of borderline leprosy are described: borderline tuberculous, mid borderline and borderline lepromatous leprosy. The spectrum varies from >3 well defined, dry, firm and rough, anaesthetic, asymmetric lesions with autonomic dysfunction (loss of hair and sweat) in borderline tuberculous leprosy towards more generalized, ill-defined, smooth, shiny and soft, non-anaesthetic, symmetrical lesions without autonomic dysfunction in borderline lepromatous leprosy.

**Lepromatous leprosy**
Lepromatous leprosy, photo Cochabamba, Bolivia
There are countless disseminated macules and/or skin nodules, with blurred outlines and sometimes joining to form larger plaques. No tendency to central healing is seen and there is no hypopigmentation although sometimes a “copper colour” is present. The infiltrated skin nodules do exhibit less or no anaesthesia, but numbness develops in the hands and feet. The skin infiltration may lead to diffuse skin thickening, chiefly of the ears, lips and forehead (“lion’s face” in LLp). In Mexico, the diffuse variety of leprosy is sometimes called “pretty leprosy” (lepra bonita) because it tends to iron out the wrinkles in the skin, restoring a youthful appearance to the patient. Infiltration of the mucosa leads to chronic rhinitis with epistaxis, septum perforation and destruction of the nasal cartilages. The tongue is thickened and there may be hoarseness. The upper incisors become loose and often drop out. There is often loss of the eyebrows (madarosis) and eyelashes. The central portion of the forehead (frontalis muscle) is more affected than the lateral portions. This sign is quite characteristic for leprosy and was first described by Monrad-Krohn. The sensory loss on the forehead can be quite marked (since the skin is relatively cool) but at the hairline, there tends to be an abrupt increase in the sensitivity to pinprick. Testicular atrophy leads to gynecomastia. The nerves are not severely thickened but involvement of the nerves is extensive, generalized, gradual and symmetrical. The consequences of this loss are evident later in the disease and sensory dysfunction, rather than motor defects are foremost. Deep tendon reflexes are preserved for a long time, which distinguishes this disease from many other neuropathies (except amyloidosis). Vibration sense and position sense remain intact for a long time. With progression of the disease, the motor branches of small nerves are invaded so that there is distal atrophy especially in the hands.

Clinical aspects, specific problems

Blindness

Blindness can occur in tuberculoid as well as in lepromatous leprosy. Blindness may be caused by:

1. Involvement of the facial nerve. This causes peripheral facial paralysis. Most often the zygomatic branch is effected and the eye can no longer close (lagophthalmia) which leads to drying out of the cornea. The patient attempts to draw the eyes upwards under the eyelids to moisten them. The lower eyelid may exhibit paralytic ectropion. Artificial tears may prevent corneal dehydration. Sometimes the eyelid needs surgical correction to prevent blindness.

2. Involvement of the supra-orbital branch of the trigeminal nerve leads to an insensitive cornea. The patient does not feel the dehydration or the presence of dust, resulting in keratitis. Artificial tears,
as used in sicca syndrome may be beneficial. The patient should be taught to consciously blink (“Think blink”).

3. Infiltration of the eye by countless bacilli with possible formation of lepromas (nodules full of bacteria). The latter obviously only occurs in lepromatous leprosy.

4. Iridocyclitis, for which eye drops with steroids are indicated. It is seen in lepromatous leprosy. Cataract formation and phthisis bulbi are late complications.

5. Beware of glaucoma in patients treated with cortisone for leprosy reactions as cortisone can increase intra-ocular pressure. Topical cortisone administered as eyedrops is more dangerous than systemic cortisone.

**Mutilations**

Mutilations may occur due to:

1. Paralysis with muscular atrophy and contractures.

2. Loss of sensitivity leads to not noticing wounds or burns and maintaining postures which would otherwise be painful.

3. Failure of autonomous nerves resulting in trophic skin disturbances. Dry skin with crack occurs.

4. Untended wounds with secondary infections may lead to chronic ulceration. Tissue destruction and bone resorption lead to mutilation of the fingers, hands, toes and feet. Most mutilations can be avoided. E.g. to avoid claw-hands, the patient should passively stretch their fingers daily.

5. Direct destruction of tissues, e.g. the nose. Bone lesions in LL are often more attributable to direct destruction than due to bacterial infiltration. Injuries are made worse by anaesthesia, superinfection, atrophy and ankylosis following disuse. The phalanges of fingers (dactylitis) and toes are most frequently affected, shorten gradually and become thinner due to lateral bone resorption.
Multibacillary leprosy. Ulcus penetrans on foot. Copyright ITM
Multibacillary leprosy, hand mutilation. Copyright ITM
Leprosy with mutilations of the feet. Bone destruction is clearly visible on this X-ray. Notice to so-called pencil shape of some affected phalanges. Copyright ITM
Leprosy reactions

The host’s immune responses to the leprosy bacillus create some of the pathology associated with the disease. Reactions in leprosy can occur before, during or after treatment, though they are most often seen in the months following treatment. Beware: A leprosy type 1 reaction has nothing to do with the hypersensitivity-anaphylactic type I reaction.

Type 1 or reversal reactions

Lesions caused by a change in the immunologic defence of the patient are called type 1 reactions. These reactions may by triggered by: treatment, pregnancy, inter-current illness or vaccination or is sometimes due to spontaneous changes in immune defence. Polar tuberculoid or lepromatous forms are generally immunologically stable and do not develop type 1 reactions (only the 3 borderline
stages are unstable). In the central part of the clinical spectrum there are fluctuations in the number of bacilli and in the patient’s resistance. If the patient’s immunity increases many leprosy bacilli will be destroyed. The body may react strongly to proteins released from these dead bacilli. This type of reaction (previously called an upgrading reaction: increase in cellular immunity; towards TT) may cause damage to the body itself. Existing skin lesions become inflamed, discoloured red and painful. [Signs of inflammation of the leprosy patches are only found in leprosy reactions]. There will rarely be new lesions. Paralysis may occur quickly with a sudden increase in size and tenderness. Treatment of such a reaction must be swift to limit the damage: anti-inflammatory therapy (aspirin, indomethacin, corticosteroids), immobilization of the affected body part and sometimes decompressive nerve surgery. The leprosy therapy is not discontinued. Side effects of steroid therapy include Cushing’s syndrome with weight gain, moon facies, steroid acne, osteoporosis, gastritis, diabetes and steroid cataract.

Sometimes the immune response may be reduced. Nowadays this is seldom seen with the combination therapy. Progressive leprosy lesions develop (previously called a downgrading reaction: decrease in cellular immunity, towards LL). They take a less dramatic course than upgrading reactions. The treatment is swift adjustment of the leprosy therapy.

**Type 2 reactions**

Patients with LL and BL have almost no cellular defence against the leprosy bacillus. They do produce many antibodies, but these are not protective. The antibodies may precipitate in the body in the form of immune complexes and cause a different set of lesions (type III hypersensitivity reaction). This type of reaction is called a type 2 leprosy reaction. It is also called Erythema Nodosum Leprosum (ENL). ENL shows a high relapse tendency. Leprosy reactions are an important cause of mutilation. These reactions appear as sudden new red painful skin nodules on the legs and arms, which may form sterile pustules or ulcers. The symptoms are usually generalized, such as fever and general malaise, accompanied by muscle and joint pain, proteinuria, inflammation of the eyes and swelling and pain in the nerves, acute epididymo-orchitis. Approximately half of LL and BL patients develop ENL a few months after beginning chemotherapy. Patients with TT are spared this complication.

Differentiation between type 1 and 2 reactions is not always easy, nor the distinction between leprosy reaction and relapse of disease. For treatment of Type I and Type II reactions see further in this chapter on treatment.
Lepromatous leprosy with leprosy reaction type 2. Copyright ITM
Lepromatous leprosy with leprosy reaction type 1 in the face. Copyright ITM

**Lucio’s phenomenon**

In 1852, Lucio and Alvarado described a necrotizing skin reaction associated with non-nodular diffuse leprosy. Lucio’s phenomenon occurs in diffuse lepromatous leprosy (Lepra bonita) and can be considered an extreme type II reaction. It occurs in untreated patients and is mainly known from Mexico and other countries in Central America. It is characterized histologically by ischemic necrosis of the epidermis as a result of necrotizing vasculitis of small blood vessels whose endothelium is massively invaded by *Mycobacterium leprae*. Clinically, one can recognize eruptions of crops of small erythematous lesions with central necrosis. The eschar may be shed, revealing ulceration, with eventual scar formation. Large painful haemorrhagic skin infarcts and vasculitis lesions can occur. The resultant ulcers are large with undermined edges and necrotic bases. Smears from the bases generally show large numbers of acid-fast bacilli. This condition is treated with wide surgical excision with skin grafts. The ulcers will not be cured by chemotherapy.
IRIS reaction in HIV patients

In the first decade of the 21st century, antiretroviral medication became more widespread available in developing countries. In the first four months of therapy there is a danger of immune reconstitution syndrome (IRIS). The rapid recovery of cell mediated immunity triggers immune response to foreign antigen. This presents with the first, often dramatic manifestation of an existing subclinical infection, or the deterioration of existing lesions. Acute reactions in leprosy lesions can result in severe skin inflammation, ulceration and rapid loss of nerve function. The patient might mistake the HAART as responsible for leprosy symptoms. Strange at first sight but prolonged immunosuppressive therapy may be necessary while the patient’s immune system recovers from the suppression by HIV.

Diagnosis

General

Lepromatous leprosy, skin biopsy. Numerous acid-fast mycobacteria are visible. They typically cluster
in globi (small groups), which is strongly suggestive for this disease.

The diagnosis of leprosy is based on clinical and microscopic examination. Leprosy cases are often cared for by specialized teams, previously more so than nowadays. The role of the first line health workers should not be underestimated: recognizing the illness, following up the patient (leprosy reactions, eyes, wounds, foot care).

There are 3 cardinal signs for leprosy diagnosis. At least 1 of the 3 must be present to make the diagnosis of leprosy in an endemic area:

- Anaesthesia over the skin lesions
- Enlargement of peripheral nerves with or without tenderness with evidence of nerve damage: loss of sensation, muscle paresis or paralysis of hand, feet or eyes
- Demonstration of *Mycobacterium leprae* in the skin smears.

**Clinical aspects**

The reason for an initial consultation is often the observation of painless traumas, burns or chronic skin abnormalities. Sometimes the initial presentation is an acute problem, e.g. ENL triggered by pregnancy, delivery, concomitant illness or vaccination.

**Skin lesions**

1. Check the texture, colour, hair growth, sweating. Anhydrosis occurs quite early due to trophic and vasomotor disturbances (chiefly in tuberculoid leprosy). Loss of hair occurs, and the skin is often atrophic. Yet oedema also occurs, even progressing to elephantiasis of the feet and legs.
2. Loss of eyebrows and eyelashes (madarosis) in lepromatous leprosy.
3. Macules, papules, plaques or nodules. A leprosy macule is never completely colourless, has never been present since birth and does not flake or itch unless there is a leprosy reaction.
4. Open wounds are complications: not primary signs.

**Diminished sensitivity (numbness)**

Examining sensitivity in a reliable manner is not easy. Use two basins with cold and hot water, a cotton wool ball, a feather, a needle. A tuning fork of 128 Hertz can be used for proprioception. One technique for testing sensitivity of the feet is to use a Semmes-Weinstein monofilament. This
monofilament is a supple thread of artificial material such as nylon, mounted on a holder. The thread is pressed perpendicularly against the foot until it assumes a C-shape. In this way a standardized pressure can be created. If the patient does not feel this, there is neuropathy leading to an increased risk of foot ulcers. When the soles of the feet are hyperkeratotic, the test is more difficult to interpret. When seeing a patient suffering from sensory loss, one has to try to detect an underlying pattern during the neurological examination. Typical patterns include:

1. mononeuropathy, when isolated damage to an individual nerve affects the sensation in the area of the nerve.
2. mononeuritis multiplex. Similar to mononeuropathy but several peripheral nerves are affected.
3. polyneuropathy in a glove-and-stocking distribution of impairment. The longest nerves tend to be involved first in metabolic or toxic causes, e.g. diabetes, alcohol.
4. dermatomal distribution. Sensory loss corresponding to the cutaneous distribution of a spinal nerve root. This shows the importance of knowing the dermatomes.
5. sequence of failure: \( t^\circ > \) fine touch > pain > deep pressure.

**Thickening of superficial nerves**

Examine and palpate peripheral nerves systematically. Some of the most important are: supraorbital nerve (above the eye socket), great auricular nerve (in the neck, arises behind the sternocleidomastoid, ascends, curving diagonally across that muscle, and courses forwards and upwards), ulnar nerve (at the elbow), median nerve (ventral side of the wrist) radial nerve (the superficial branch at the wrist), lateral peroneal nerve (the knee, at the head of the fibula), posterior tibial nerve (behind the medial malleolus) and near a skin lesion.

**Neural dysfunction**

1. Painless wounds, risk of burns due to the lack of pain sensation.
2. Peripheral facial paralysis with the risk of eye lesions due to lagophthalmia with drying of the cornea.
3. Trigeminal nerve involvement with risk of eye lesions due to insensitivity of the cornea. Test with cotton wool stick.
4. Atrophy of the thenar (common digital nerve) and of the hypothenar eminence.
5. Claw hand with atrophy of the interossei (ulnar nerve). Reminder: there are seven interosseous muscles, 3 palmar (adduction of fingers) and 4 dorsal (abduction of fingers). They assist the lumbrical muscles to bend the metacarpophalangeal joints and to extend the interphalangeal joints. They are all innervated by the ulnar nerve. A good clinical test for these muscles is to spread
and then adduct the fingers. A sheet of paper between the adducted fingers must be firmly held. Froment’s sign tests for palsy of the ulnar nerve, and more in specific the action of adductor pollicis. To perform the test, a patient is asked to hold a piece of paper between the thumb and his flat hand palm. The paper is then is pulled away. If the ulnar nerve is intact, the patient will be able to maintain a hold the paper without difficulty. In case of ulnar nerve palsy, this will be difficult. The patient might compensate by flexing the flexor pollicis longus of the thumb (flexion of the DIP joint of the thumb), a muscle innervated by the median nerve.

6. Opposition of the thumb. If the median nerve is affected, the m. abductor pollicis brevis, the m. flexor pollicis brevis and the m. opponens pollicis become dysfunctional and opposition of the thumb is compromised.

7. Wrist drop (radial nerve). Dorsal wrist extension is weak or not possible.

8. Foot drop (fibular nerve = peroneal nerve). Heel gait is not possible.

9. Claw toe (paralysis of flexors) and loss of sensation at sole of foot (posterior tibial nerve). The patient cannot walk on his or her toes.

10. Painful peripheral neuropathy can also occur: leprosy is not always a painless disease! Gabapentin and especially pregabalin (Lyrica®) are useful against neuropathic pain. Pregabalin is active on calcium channels that play a central part in neuropathic syndromes. In general, pregabalin is preferred above tricyclic antidepressants and anti-epileptic drugs.

**Electromyography**

Nerve-conduction studies provide two basic measurements. The first is of the total number of units that respond on either the motor or the sensory side. The total sensory or total motor potential (sensory action potentials and motor action potentials) indicates the number of axons that have reached their destination and are still functioning. In axonal neuropathies, such as those due to vincristine, alcoholism, diabetes or uraemia, an early reduction in sensory action potential is recorded from the distal parts of the extremities. Amyloid neuropathy gives similar results. The second measurement is of the conduction velocity which reflects Schwann-cell or myelin function. It is a measurement of preservation of saltatory conduction down the nerve fibre. A few diseases affect primarily myelin in the peripheral nerves, e.g. Guillain-Barré syndrome and its variants. Chronic pressure, such as in carpal tunnel syndrome, leads to pressure lesions and can result in prominent slowing of the conduction velocity. Mixed lesions are common. In leprosy, conduction velocities are reduced in a spotty fashion.
Microscopy

Acid fast bacilli in smear

Preferably several smears should be taken from the ear lobes, forehead, chin, buttock and from the raised edge of active skin lesions. The latter is sometimes forgotten. The skin is pinched between the thumb and finger of one hand. Make a small incision with the other hand (5 mm long, 2 mm deep) using a scalpel, scrape a little tissue away which is then smeared onto a slide (slit-skin smear). Try not to include any blood in the smears. Smears are also sometimes made from nasal mucosa.

The smears are stained with a modified Ziehl-Neelsen stain (e.g. Kinyoun stain). It is a cold stain. Discoloration is with a low concentration of acid (1% HCl). The mycobacteria are less acid-fast than *Mycobacterium tuberculosis* and bleach too much with standard Ziehl-Neelsen, which uses more concentrated acid. *M. leprae* is a weak Gram-positive or Gram-neutral acid-fast bacillus measuring 0.3×2-7 µm. The bacteria are often lying grouped in clusters (globi). Probably the hydrophobic character of the waxy mycobacterial capsule plays a part here.

The morphological index has increasingly been abandoned. It is the percentage of live bacteria in relation to the total number of bacteria. For this, 200 free-lying bacteria are examined. Live bacteria are homogeneously stained. Dead bacteria have a granular staining pattern. The resorption of dead bacilli into the tissues is very slow (1 log decrease per year). The presence of acid-fast bacilli in a treated patient does not necessarily mean that the therapy has failed. The morphological index is a better measure of recovery than the bacterial index. The disappearance of bacteria during treatment can be partly attributed to the loss of their acid-fast nature. In some biopsies which test negative with the Fite-Faraco stain, bacteria can still be detected using Gomori methenamine-silver staining.

The bacterial index was proposed by Ridley. He developed a logarithmic scale, from 0 to 6+. The scale is based on the average number of bacilli per microscopic field using an oil-immersion objective. In infections with a high bacterial load, it usually takes 5-8 years from the beginning of therapy before the bacterial index is negative. [A rule of thumb is 1+ per year].

<table>
<thead>
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<th>Bacterial index</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 bacilli in 100 oil-immersion fields</td>
</tr>
<tr>
<td>1+</td>
<td>1 to 10 bacilli per 100 fields</td>
</tr>
<tr>
<td>Level</td>
<td>Description</td>
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<tr>
<td>-------</td>
<td>-------------</td>
</tr>
<tr>
<td>2+</td>
<td>1 to 10 bacilli per 10 fields</td>
</tr>
<tr>
<td>3+</td>
<td>1 to 10 bacilli per field</td>
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<tr>
<td>4+</td>
<td>10 to 100 bacilli per field</td>
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<tr>
<td>5+</td>
<td>100 to 1000 bacilli per field</td>
</tr>
<tr>
<td>6+</td>
<td>&gt; 1000 bacilli per field</td>
</tr>
</tbody>
</table>

**Biopsy**

If the smears are negative, a skin biopsy is performed, which must penetrate the subcutaneous tissue. The biopsy should preferably contain a superficial nerve branch (hypodermic). In multibacillary leprosy there is a zone of healthy tissue between the superficial epidermis and the infiltrate with bacilli in the deeper dermis. This does not apply to tuberculoid leprosy. The linear distribution of an infiltrate consisting of neutrophils and vacuolated macrophages follows nerves and blood vessels. The foamy characteristic of the histiocytes are an important clue. Bacilli in a tissue biopsy can most easily be detected with a modified Ziehl stain (Fite-Faraco stain). *Mycobacterium leprae* stains poorly with Ziehl on sections from paraffin tissue blocks. The acid fastness may be restored by impregnating the tissue segments beforehand with peanut oil or turpentine. In TT it will rarely if ever be possible to detect acid-fast rods, but the diagnosis can be made on the basis of typical histological appearance (non-caseous granulomas around a nerve branch). In indeterminate leprosy there is minimal non-specific chronically inflamed infiltrate around nerves, blood vessels and skin nodes. Bacilli are very seldom present in indeterminate leprosy and generally none are found. A sural nerve biopsy can be performed in cases where the diagnosis is not clear. The presence of neutrophils and oedema of the papillary dermis is also an important clue to a Type 2 reaction. Type 1 reactions by contrast are lymphocyte rich.

**Lepromine**

Lepromine is not used as a diagnostic aid for the individual patient. In the time before armadillos could be used, lepromine was prepared from skin nodules from multibacillary patients. It was called lepromine H (human), with 160 million bacilli/ml as standard. At present lepromine is prepared from armadillos and is known as lepromine A (armadillo). Both human and armadillo lepromine contain varying amounts of tissue. A more purified preparation is Dharmendra
Lepromine, which is used in India. This produces a weak Mitsuda reaction. Sometimes another purified version is used, called Convit’s soluble antigen.

**LTT**

Lymphocyte transformation tests have been developed. They are more specific than lepromine tests. It was observed that many healthy people who had had contact with lepers reacted positively in this test, unlike people who had not been exposed to leprosy. This is an argument for the hypothesis that leprosy is very infectious but has a very low disease-expression.

**Serology**

Using serology, antibodies can be detected but this produces many practical problems. One of the better studied antibodies is called phenolic glycolipid-I antibody (PGL-I). The titres is proportional to the bacillary load. Newly lepromatous patients are always positive, but the diagnosis can be reached in a simpler way. Up to 50% of tuberculoid patients are negative in this test. At present the technique (ELISA or dipstick assay) is more and more abandoned. Leprosy sufferers often have circulating auto-antibodies so that their plasma often give false-positive results for various other disorders (e.g. positive RPR or VDRL suggesting syphilis). Cross-reactivity with Leishmania is described, which is an important differential diagnosis in regions where both diseases are endemic. The cerebrospinal fluid in leprosy is normal.

**PCR**

A PCR [polymerase chain reaction] has been developed to test for *M. leprae*. In view of the inherent problems with this technique due to contamination in the laboratory, the results from various studies must be interpreted with caution. In many people positive PCR results from nasal swabs (e.g. 33% in contact persons in the same household and 20% of persons who work with leprosy sufferers) are found. Positive PCR results must therefore be confirmed independently. It is possible that there are indeed many asymptomatic carriers.

**Culture**

If there is doubt concerning resistance, an in-vivo culture can be carried out in research centers (injection in the food-pad in mice). The inoculated test animals then receive food mixed with various concentrations of dapsone, rifampicin or clofazimine. Maximum growth of the resistant
bacteria is reached in approximately 6-9 months. To bypass the problems associated with experimental animals, attempts are being made to develop in-vitro techniques. Using in-vitro radiorespirometry (\(^{14}\text{CO}_2\) production from \(^{14}\text{C}\)-labelled palmitinic acid) as in the Bactec or Buddemeyer systems, an attempt can be made to measure the metabolism of the bacteria, and in future it should be possible to use this to examine the viability of the mycobacteria, e.g. during treatment. These techniques have no place in daily clinical practice. It must be stated that no long term in-vitro cultivation technique is available.

**Differential diagnosis**

Initially the differential diagnosis must take into account a large number of other diseases. Fixed drug eruption, morphea (localised scleroderma), dermatophytosis, dermal filariosis, eczema, scars, nodular cutaneous leishmaniasis, post-kala azar dermatitis and keloids may exhibit clinical similarities.

**Diffuse cutaneous leishmaniasis** often resembles lepromatous leprosy and can be similar to cutaneous lymphoma (mycosis fungoides).

**Lobomycosis** or Lobo’s disease is very rare and occurs almost exclusively in the Amazon and Orinoco basins, although some cases have been known from Surinam and Central America. The disease is caused by a fungus, *Loboa loboi*, and may be clinically very similar to lepromatous leprosy or keloids. The diagnosis is with a skin biopsy.

**Systemic lupus erythematosus** (SLE) may be mistaken for leprosy. Skin and mucosal lesions of lupus erythematosus discoides, necrobiosis lipoidica (check for hyperglycaemia) and of porphyria cutanea tarda (lesions chiefly on the hands and face, where exposed to the light) may pose diagnostic problems.

**Neurofibromatosis** (Recklinghausen’s disease) sometimes causes a problem in differential diagnosis. In neurofibromatosis type 1, 100% of the children have café au lait patches before they are 2 years old, 70% have freckles in the skin folds (axilla) and 90-100% of patients also have hamartomas in the iris (Lisch’s nodules) as well as neurofibromas by the time they are 20 years old. In the rarer type 2 (NF2) café au lait patches only occur in 1% and the freckles are absent. In NF2 the peripheral nerves may develop schwannomas, but in these patients acoustic neurinomas are the most common.

**Annular skin lesions** which are similar to tuberculoid leprosy may also occur in tinea corporis, cutaneous sarcoidosis (lupus pernio), granuloma annulare, granuloma multiforme, syphilis, actinic
granulomas and Jessner-Kanof’s lymphocytic skin infiltration (pseudolymphoma; aetiology unknown). A Sutton’s naevus is generally easy to recognise (ring-shaped depigmentation with central hyperpigmentation).

**Annular psoriasis** is characterised by the presence of thick scales which usually exhibit symmetrical distribution, with enlarged blood vessels in the dermis. There may be pustules or pitting of the nails and/or arthropathy. Köbner’s phenomenon may occur.

**Granuloma annulare** is more difficult to differentiate. It is a benign skin disorder characterised by a granulomatous inflammatory process, which manifests itself in a ring or annular configuration of papules. The lesions usually occur in the region of a joint (the hands, elbows), but may also occur elsewhere. There is no neural dysfunction. An aetiological association with sunlight is assumed, but this is only one hypothesis. Most lesions (75%) heal spontaneously in 1-2 years. It is possible that granuloma multiforme is a variant of granuloma annulare. Biopsy is usually central to the diagnosis. In view of the strong similarity to leprosy and since granuloma multiforme is regularly seen and treated as leprosy, it is advisable to study a number of photographs of people with this disorder, or better still the patients themselves, in order to become familiar with the clinical picture.
Systemic lupus erythematosus with butterfly rash on the face. This patient was wrongly diagnosed as having leprosy and treated as such for one year before the correct diagnosis was made. Photo prof Gigase, copyright ITM.

**Pityriasis alba** is an eczema variant (slightly scaly, on skin exposed to the light). Gibert’s pityriasis rosea is another condition which is easier to differentiate.

**Pityriasis versicolor** (Gr. “pityron” = bran; refers to the light skin scaliness) is a very common skin infection with a fungus: *Pityrosporum ovale* (yeast stage) or *Malassezia furfur* (mycelium stage). This lipophilic fungus forms the tyrosinase inhibitor azelaic acid from sebaceous fats, a substance which inhibits melanin synthesis. This explains the white appearance of the skin spots. Account must be taken of the fact that depigmented skin spots can also be caused by damage to the melanocytes (pigment cells) after an ordinary infection, wound or burn (post-inflammatory hypopigmentation).

**Vitiligo** is easy to differentiate cause mostly depigmentation is complete (never complete in leprosy) and the texture of the skin with this condition is otherwise normal.

**Endemic treponematosis and syphilis** (the differential diagnosis is often difficult here). It is important to know that people with leprosy often have a false positive VDRL (screening for syphilis). TPHA [the *Treponema pallidum* haemagglutination test] permits differentiation.

**Trichoepithelioma** is a condition resembling leprosy with numerous, rounded, skin coloured firm papules and nodules. It is a benign tumour originating in the hair follicles.

There are not many neuropathies where temperature and pain sensation are diminished, while sparing vibration and position sense, as well as sparing deep tendon reflexes. One should consider in these cases **primary amyloidosis** and **syringomyelia** (lesion of the crossing fibres of the central grey matter of the spinal cord) in the differential diagnosis. Less than 10% of leprosy cases develop secondary amyloidosis. Patients with primary / hereditary amyloidosis usually have pronounced autonomic neuropathy from the onset, with episodic diarrhoea, impotence, decreased sweating, postural hypotension and other evidence of impaired vasomotor control.

**Therapy**

Because of the increasing resistance to dapsone, in 1982 the WHO proposed to use only combination regimens. With modern therapy the infectivity falls very swiftly (a few weeks). People are being cared
for more and more in their normal environment. This requires huge efforts in follow-up. Rehabilitation, orthopaedic aids, good shoes and eye care are very important. Surgical reconstruction, tendon transplantations etc. have their place, but require specialized physicians. The instruction of patients, chiefly concerning checking wounds and foot hygiene, is very important. Prompt treatment of wounds can prevent much suffering.

**Historical note**

In the past, leprosy sufferers were strictly avoided or isolated in a leprosarium. This completely disrupted the social lives of the people affected. Patients hid themselves and withdrew from care.

Dapsone was first synthesised by Fromm and Whitmann in 1908, but it was used exclusively in veterinary medicine for streptococcal mastitis. In 1941 it was discovered that Promin® (sodium glucosulphone) PO and IV could produce an improvement in leprosy. Diasone, another sulphone, was better tolerated but was later replaced by dapsone. The first cases of dapsone resistance were reported in 1964. In the ‘60s the efficacy of clofazimine was discovered. In 1965 the activity of thalidomide in ENL was ascertained. In the late ‘60s and early ‘70s rifampicin was developed and this exhibited exceptional efficacy.

**Dapsone**

The anti-leprosy activity of this sulphone was ascertained in the ‘40s and until 1980 it was often used in monotherapy (initially IM, later PO). It is safe during pregnancy. Dapsone (=DDS; Diamino Diphenyl Sulphone) is a slow-acting bacteriostatic product. It is swiftly absorbed from the intestine and undergoes enterohepatic circulation. A steady-state serum concentration is reached approximately eight days after beginning the treatment. It has a half-life of 28 hours and can be taken once daily. Dapsone resistance is presently spread world-wide. Dapsone is generally well tolerated.

1. Pharmacological predictable adverse reaction to dapsone

- peripheral neuropathy
- haemolytic anaemia (even if there is no G6PD deficiency)
- methaemoglobinemia
- nonspecific nausea, vomiting, fatigue, dizziness, weakness, headache
- Allergic / idiosyncratic reaction: the dapsone hypersensitivity syndrome. This usually starts within 6 weeks after beginning dapsone. If there is no alternative, desensitisation may need to be carried
out. Symptoms:
- hepatitis with icterus
- eosinophilia
- fever
- skin eruption including exanthema, pustular lesions and even Stevens-Johnson syndrome
- lymphadenopathy
- agranulocytosis
- nephritis
- pneumonitis
- hypothyroidism

Other medical uses of dapsone
Dapsone is also used in the treatment and prevention of Pneumocystis jirovecii, in the treatment of toxoplasmosis, in dermatitis herpetiformis, in Loxosceles bites (see the chapter “spiders”) and several other rare disorders. Dapsone is contained in Lapdap® and Maloprim®, agents for malaria prophylaxis.

Rifampicin
Rifampicin (Rifadin®, Rimactan®) (id. Rifampin) is a highly active but expensive bactericidal agent. It interferes with the synthesis of nucleic acids by inhibiting DNA dependent RNA polymerase. Due to its high sterilizing activity and the slow growth of M. leprae it can be given once monthly if combined with other drugs. This reduces the cost and toxicity significantly without compromising efficacy and makes supervision of adherence easier. Rifampicin sometimes causes liver damage. See also tuberculosis. It may be used during pregnancy, although there are isolated reports of congenital deformities. Spina bifida and hare lip were observed in the progeny of rodents when the product was administered at high doses during pregnancy.

Clofazimine (Lamprene®)
Clofazimine is a weak bactericidal agent. It has anti-lepromatous and anti-inflammatory properties. This lipophilic drug is best taken after a meal for better absorption. It accumulates slowly in the skin, where it may cause dryness and red discoloration. The latter may sometimes cause difficulties in white patients. The urine, tears and sweat are also stained red. Sometimes there is nausea. In rare cases there is severe enteritis with paralytic ileus. The tissue half-life is very long (70 days). If
Clofazimine is used in type 2 leprosy reactions, the effect is usually only noticeable after 4 to 6 weeks. Clofazimine passes the placental barrier and is present in breast milk. Neonates may then also exhibit hyperpigmentation. It is probably safe during pregnancy.

Other

In 1987 it was discovered that minocycline, ofloxacin (Tarivid®) and clarithromycin (Biclар®) possess bactericidal properties against Mycobacterium leprae. The therapeutic place of all these drugs in the treatment of leprosy still needs to be determined. They may be used if for example rifampicin is not tolerated. Shorter therapies (single dose and 6 weeks) are being studied but have been abandoned due to too many failures on follow up.

Ansamycin (Rifabutine®) is said to be beneficial in rifampicin resistance. Ethionamide (Trecator®) and protonamide (Trevintex®) are moderately bactericidal agents which are still not widely used (250-500 mg/day). Thioacetazone (= thiosemicarbazone) is a weak bactericidal agent, little used in this indication.

Typical regimens

Paucibacillary leprosy (TT and BT)

For 6 months

Rifampicin 600 mg/once per month under supervision

Dapsone 100 mg/day without supervision

Then keep under supervision for a further 2 years, for late leprosy reactions and any relapse.

Multibacillary leprosy (smear-positive BT, BB, BL and LL)

For 1 year (in some projects longer if the BI > 4+)

Rifampicin 600 mg/once per month under supervision

Clofazimine 300 mg/once per month under supervision
Clofazimine 50 mg/day without supervision

Dapsone 100 mg/day without supervision

Then keep under supervision for a further 5 years (or life-long in LL).

These regimens are usually quickly accepted and have little toxicity. Relapses seldom occur (< 5% after several years).

**New experimental regimens**

Brief therapies with single dose Rifampicin-Ofloxacin-Minocycline ± Clofazimine ("ROM" and "ROMC") have been used for both single skin lesion paucibacillary leprosy (SSLPL). More recent data point to higher failure rates and this short-course treatment is more and more abandoned.

**Pregnancy and lactation**

There is very little data on leprosy and pregnancy. During pregnancy there is progressive reduction of the cellular resistance but humoral immunity is probably stimulated. In theory fewer type 1 reactions would be expected during pregnancy. On the other hand, type 2 reactions may be more frequent. Possibly there is an increase in the bacillary load in untreated patients. Since the disease may become worse during pregnancy, the medication is continued unchanged. The use of thalidomide during pregnancy is of course forbidden.

**Neuropathic pain in leprosy**

Leprosy patients often suffer from neuropathic pain. Carbamazepine (Tegretol®) can be used but can result in a lupus-like syndrome. Pregabalin (Lyrica®) is approved for chronic neuropathic pain (leprosy, diabetes, shingles). It can be administered orally, for example 150 mg in the morning and 300 mg in the evening.

**Treatment of leprosy reactions**

Treatment of type I leprosy reactions

Treatment of such a reaction must be swift to limit the damage: anti-inflammatory therapy (aspirin, indomethacin, corticosteroids), immobilization of the affected body part. The leprosy therapy is not
discontinued. Contrary to tuberculosis, prolonged steroid use does not seem to increase the risk for severe leprosy nor to re-activate asymptomatic infections.

**Treatment of type II leprosy reactions with erythema nodosum leprosum**

The treatment of ENL consists of analgesics, clofazimine (which also has anti-inflammatory characteristics) at higher doses than normal leprosy therapy (100 mg 3 times daily for 1 to 3 months) but it is a slow-acting drug, corticoids systemically and if necessary eye drops. If needed the fast-acting drug thalidomide can be used (Softenon®, 100 to 400 mg/day for 10 days, then reduced to 50-100 mg daily). **Methotrexate** seems a good alternative in patients with poor response to steroids that cannot take thalidomide or had poor response to thalidomide.

Thalidomide is not an immunosuppressive, but is immune-modulating drug. It changes the balance of several cytokines. For example, it is an antagonist of TNF-alpha and increases the action of IL-2. Contraception is mandatory during the use of thalidomide (men and women), since it is highly teratogenic, probably due to interference with angiogenesis in the fetus, not due to induction of mutations. It causes phocomelia, heart, ear and eye abnormalities, autism and embryopathy. Thalidomide was officially taken off the market in 1961. In 1965 Dr Jacob Sheskin, an Israeli dermatologist discovered fortuitously that thalidomide in leprosy patients improved ENL. In 1998 thalidomide was approved by the FDA for treatment of ENL and in 2006 for treatment of multiple myeloma.

Thalidomide is now used in erythema nodosum leprosum and in a number of immunologically mediated diseases, such as refractory mucosal aphthosis (common in AIDS), Behçet’s syndrome, severe erythema multiforme and severe prurigo nodularis (Hyde’s disease). Apart from teratogenicity, side-effects include peripheral neuropathy (risk higher when cumulative dose is greater than 20 grams), somnolence, constipation, nonspecific skin rash and dizziness.

**Lenalidomide**

Lenalidomide (Revlimid) is a 1:1 racemate and chemically related to thalidomide. It is studied in myelodysplastic syndromes. About two thirds of patients with the 5q- syndrome (myelodysplasia with anaemia and thrombocytosis) benefit from lenalidomide. At present it is used in Kahler’s disease (multiple myeloma). It might replace thalidomide in the future for certain indications. Lenalidomide is 50,000 times more potent than thalidomide in inhibiting tumour necrosis factor-alpha. Because of its resemblance to thalidomide, it is contra-indicated in pregnancy. It’s place in leprosy reactions is not yet clear today.
Cyclosporin A in leprosy

Cyclosporin A acts primarily to suppress T-cell activation, especially the CD4-Th1 helper cell, which play a central role in reversal reactions. Such reversal lesions contain high numbers of CD4-lymphocytes, especially Th-1 helper cells. This is in contrast with ENL, where an influx of CD4-Th2 cells and deposition of immune complexes occurs. Prednisone remains the drug of choice in reversal reactions, but in case of failure, cyclosporin A can be used as an alternative.

Prevention

Basic hygiene is important for staff and patients alike: washing hands, wearing a mask if the patient has rhinitis, gloves to take samples. *Mycobacterium leprae* is found in breast milk, but this is not sufficient reason to stop breast feeding. It is thought that infectivity quickly drops to zero after the start of combined chemotherapy. Examination of the people in contact with leprosy patients is indicated. The risk of leprosy in the family of lepromatous patients is 5-8 times higher than in the general population. Previously high figures were recorded in lepersaria (in 1930 up to 23% of the children born in these institutions). In the case of contact with multibacillary patients, check-ups for 5 to 7 years are preferable, once per year (including looking for the “numb spot”). Chemoprophylaxis of contacts (rifampicin) is not advised at present. The higher the socio-economic status of a country, the lower the incidence of leprosy (regardless of any leprosy control programs).

Due to the complex bacterial cell wall combined with the difficulty to cultivate *M. leprae* in vivo only, no good antigen for vaccine production has been found today. BCG vaccination provides partial protection.

Miscellaneous skin deseases
Buruli ulcer

Summary

- Skin ulcers caused by *Mycobacterium ulcerans*
- Role of mycolactone, the Buruli toxin secreted by the organism
- Extensive involvement of subcutis and underlying tissue
- Little pain
- Surgical intervention is the first choice for treatment
- Add rifampicin plus streptomycin if early diagnosis/lesion

Historical note

In 1897, a disease was noted in Africa by Sir Albert Cook that is most likely to have been Buruli ulcer. Between 1923-35 the condition was also observed by Kleinsmidt in north-east Congo. The disease was seen in 1940 and subsequently (1948) described by MacCallum in Australia as Bairnsdale ulcer. Afterwards similar ulcers were found in Africa, Papua New Guinea and other parts of the world. In 1961 a focus was discovered in Uganda along the White Nile in Buruli County near Lake Kyoga, hence the name Buruli ulcer which has since been used extensively. After 1980, important new foci were discovered in West Africa. Since December 1997, the condition has been recognised by the WHO as an important emerging disease. The “Global Buruli Ulcer Initiative” was launched in February 1998 with the intention of improving knowledge and control of this disease.

Geographical distribution

The geographical range of the disease is still incompletely known. In the year 2000, the condition was known to occur in:

- Benin, Burkina Faso, Cameroon, Ivory Coast, Ghana, Guinea, Liberia, Nigeria, Sierra Leone, Togo, Angola, Congo, Gabon, Sudan, Uganda

- Australia, Papua New Guinea

- China, India, Indonesia, Japan, Malaysia

- Bolivia, French Guyana, Mexico, Peru, Surinam
Aetiology

Buruli ulcer is caused by *Mycobacterium ulcerans*, an organism that is closely related to *M. tuberculosis*. These bacteria are acid-fast rods, 3-7 µm long. The generation time is 20 hours (slow-growing organism). The reservoir and the route of transmission remain unknown. Regular reference is made to the presence of the disease in marshy areas along large rivers. *M. ulcerans* grows best at low oxygen concentrations, such as are found in the mud of marshy ground. The clinical history often includes a report of minor trauma, an insect bite or a hypodermic injection at the site of the original solitary lesion. It is suspected that transmission might occur via the bite of infected water bugs. These insects are possibly infected by filter feeding on micro-organisms in the water, subsequently serving as mechanical vectors. This however is still only a hypothesis. The mycobacteria are detectable in those insects by PCR. Mosquitoes were suspected in a large outbreak in Australia (PCR-positive). As a rule, attempts to isolate the organism from the environment (e.g. streambeds of slow-flowing rivers or marshes) fail. The interval between sampling and culture, the transport media, the temperature and the aggressive decontamination procedures that are used possibly play a part in this.

Pathology

*M. ulcerans* is a mycobacterium that grows extracellularly in the human body. The earliest lesion is a necrotic zone in subcutaneous fatty tissue. There is typically surprisingly little inflammatory reaction in the surrounding tissues. Clumps of acid-fast bacilli are found in the necrotic fatty tissue (“steatonecrosis”), sometimes in huge numbers. Calcifications can also form. Eventually the lesion ulcerates as a result of necrosis of the overlying skin. Necrosis of the fatty tissue is always more extensive than the ulcer itself so that the edges are undermined and become detached over a considerable distance. Multiple ulcers can form, connected at the deeper level by necrotic subcutaneous channels. From the edges of the ulcer there is a tendency to re-epidermalisation of the lowest level of the detached skin, which is pathognomonic for this disease. The base of the ulcer is coated with a layer of necrotic, purulent material in which for the most part no *M. ulcerans* is found. In contrast to tropical ulcers, these ulcers show no tendency to malignant degeneration.

The tissue necrosis extends further than the colonies of acid-fast rods. Following injection in experimental animals, a sterile ultrafiltrate of *M. ulcerans* can cause lesions that are very similar to Buruli ulcers. A cytotoxic necrotic toxin that is responsible for the steatonecrosis is found in the culture medium of *M. ulcerans*. This substance probably also has a bacteriostatic effect, which would explain the rarity of secondary infection. The toxin is a polyketide macrolide: mycolactone (C₄₄H₇₀O₉). *M. ulcerans* strains that produce no mycolactone are avirulent to guinea pigs. Mycolactone is probably locally immunosuppressant.
Clinical Aspects

Infection with Mycobacterium ulcerans. Subcutaneous lesion on arm. There is no break-through (yet) to the surface. Copyright ITM
Buruli ulcer results from infection with *Mycobacterium ulcerans*. Notice the undermined edges.

It is estimated that the incubation time is 6 weeks or longer. The ulcers are predominantly found on the limbs, more above the elbow and knees, but in 10% of cases it can be found the trunk and the abdominal wall and very rarely on the face or scalp.

The disease course can be divided into 4 stages: nodule, cellulitis, ulceration, scar. It begins as a pruritic, painless or slightly painful subcutaneous swelling that gradually becomes attached to the skin. A papulonecrotic or vesicular lesion then appears on the skin that progresses to an open ulcer with a gelatin-like coating. The skin around the ulcer is dark, sometimes with slight desquamation or with a deep reddish-purple colour (Caucasians) or hyperpigmentation (darker skin). The edges are slightly raised and rolled. The undermining of the wound edges can be established by probing. Satellite lesions and metastatic lesions in the skin or bone sometimes occur. In addition, there can be numerous lesions at the time of the first examination. The general state of health remains excellent, without fever or malaise, irrespective of how extensive the ulcer is.

When the ulcer is finally formed, it remains and becomes generally painless unless a secondary infection is involved. Sometimes localised pain is present. At the deeper level muscle, bone and joint tissue are destroyed with the accompanying formation of sequesters. Calcifications can be detected radiologically:

- in any lesion, irrespective of its location or whether it is ulcerative.
- in the skin near a lesion either before ulceration or in the subsequent scars.

In the long-term, after months or years, the ulcer tends to heal, but extensive deformities, ankylosis or lymphoedema remain. The scars are reminiscent of old burns or the consequences of late treponematoses.

**Diagnosis**

The diagnosis of the ulcerative form is somewhat easier than that of the non-ulcerative form. The undermining of the wound edges is a characteristic of Buruli ulcer. Radiologically, subcutaneous fat calcifications and/or osteomyelitis are observed in a large percentage of patients.

The acid-fast rods are examined with Ziehl stain in smears of curettage products from the edges of
the ulcer (preferably from the underside of the skin edges and not from the centre of the ulcer). The Ziehl stain of a smear demonstrates bacilli in ± 75% of cases. The histological features on biopsy are characteristic on condition that the sample has been taken sufficiently deeply to include the necrotic fatty tissue. Punch biopsies are usually not sufficient. Serodiagnosis is still experimental. Culture is possible but slow (several months). The organism grows optimally at 32°C. Higher temperatures inhibit the organism (important when transporting). Semisolid transport media such as PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin) can be used, although growth is not always obtained. The organism cannot be frozen although storage at 4°C is possible. Löwenstein-Jensen medium is best used as a culture medium in an atmosphere with little oxygen. Additionally, clinicians with Buruli ulcer experience state that the ulcers have a characteristic unpleasant smell, which can contribute to the diagnosis.

There are a few other non-tuberculous mycobacteria that can cause skin abscesses and ulcers, e.g. *Mycobacterium avium intracellulare* in AIDS patients, as well as *M. szulgai, M. terrae, M. fortuitum, M. chelonae, M. malmoense* and *M. xenopi*. *M. abscessus* is a fast-growing organism that can cause tissue necrosis after accidental contamination of a deep inoculation (injection). Of course tuberculosis and leprosy need to be ruled out.

Infection induces cross sensitivity with tuberculin. It is possible that the opposite is also true, and that tuberculosis provides partial protection against *Mycobacterium ulcerans*. Patients with active lesions often have no local skin reaction after injection of *M. ulcerans* antigen (burulin). After recovery they test positive (cell immunity).

There are various PCR methods for detecting *M. ulcerans* but the technique is expensive and only available in a few places. False positive results can be reduced by developing a meticulous technique. False negatives (e.g. as a result of the presence of PCR inhibitors) are detected by carrying out simultaneous controls with known positive samples.

**Buruli ulcer, differential diagnosis:**

1. Cutaneous tuberculosis: scrophulus, lupus vulgaris
2. Atypical mycobacteriosis e.g. Swimming pool granuloma (*M. marinum*), *M. abscessus* (post-surgery or deep injection), *M. avium-intracellulare* in AIDS-patients
3. Leprosy (less ulceration)
4. Cat scratch disease
5. Tropical ulcer
6. Tertiary syphilis (gumma)
7. Framboesia (= Yaws = Pian): Treponema pertenue
8. Rat-bite fever or sodoku: Spirillum minus
9. Ecthyma: Streptococcus pyogenes, β-haemolytic (also known as Group A Strep)
10. Cutaneous diphtheria
11. Actinomycosis or mycetoma (incl. phycomycosis), deep mycosis: histoplasmosis, blastomycosis, chromomycosis, maduramycosis, sporotrichosis
12. Cancrum oris (= Noma)
13. Cutaneous leishmaniasis
14. Cutaneous amoebiasis (Acanthamoeba, Entamoeba histolytica)
15. Pyogenic abscess with e.g. pyomyositis
16. Fistula of classic osteomyelitis
17. Trauma, residual foreign body and burns, decubitus
18. Cancer: spinocellular carcinoma (also secondary to chronic ulcer), Marjolin ulcer, Kaposi, melanoma, basocellular
19. Arterial, diabetic or venous ulcer
20. Haematological abnormalities, e.g. sickle cell anaemia
21. Vasculitis (leukocytoclastic, Behçet, microscopic polyangitis, Churg-Strauss, cryoglobulinemia)
22. Pyoderma gangrenosum. This can be difficult to distinguish from Buruli. Both have undermined edges. Pyoderma gangrenosum is often secondary to chronic inflammatory conditions such as ulcerative colitis, Crohn’s enteritis, rheumatoid arthritis, pulmonary abscesses, paraproteinemia. Acid-fast bacilli will be absent of course and the infiltration will be mainly neutrophilic.
23. Botryomycosis: S. Aureus or other bacteria
24. Inoculation chancre: tryponomiasis, rickettsia (tache noir)
25. Dracunculiasis (Guinea worm)
26. Anthrax
27. Tularemia
28. Snake bite (viperidae)
29. Loxosceles bites (spider)

**Prognosis**

The prognosis is unfavourable because of the severe skin and bone lesions, scars, tendency to infectious metastases and the problems of surgical treatment. Many lesions heal spontaneously, although with severe sequelae.
Treatment

Drug treatment is disappointing in the late stages. In vitro *M. ulcerans* is susceptible to rifampicin, clarithromycin, amikacin and streptomycin. Cycloserine, dapsone and clofazimine are active, but the organism is resistant to isoniazid. Clinical results however are often disappointing, possibly because the antibiotics do not diffuse to the bacillus itself. Treatment therefore is principally surgical: excision of the tissue followed by curettage, followed by immobilisation in a functional position. In most cases, excision of the tissues is carried out under broad-spectrum antibiotic cover. The previously mentioned antimycobacterial antibiotics can be administered at the same time to prevent the emergence of metastatic lesions. The combination rifampicin, clarithromycin and amikacin is practical. Studies suggest that an antimicrobial regimen of rifampicin plus streptomycin may be effective against early forms of Buruli ulcer. After the formation of healthy granulation tissue, skin transplants are applied (split skin grafts). Amputation may sometimes be the only possible treatment. Tetanus vaccination should not be overlooked. Good results can be obtained with local thermotherapy by surrounding the ulcer with water bottles at 40°C. This can cause logistical and technical problems. Healing of ulcers is obtained after an average of 41 days. There is little experience with hyperbaric oxygen therapy. Intensive physiotherapy can improve the function of a mutilated limb. Relapse of Buruli ulcer is not exceptional. Follow-up is important to rapidly identify those cases. Delays in seeking medical advice can lead to severe complications, including dissemination of disease and especially the development of bone lesions.

Prevention

In two studies in Uganda, BCG vaccination was shown to have about 50% efficacy against *M. ulcerans*. Protection nevertheless was temporary, on average lasting only a year. The ulcers that developed in vaccinated patients were smaller than those in controls. Possibly this merely involves non-specific immunostimulation by BCG.

Last updated by admin on July 18th, 2022

Mycobacterium marinum
Mycobacterium marinum (M. balnei) causes swimming pool granuloma. The condition was first described in Sweden and was later observed in most Western countries. It involves papules with central ulceration which heal spontaneously after a few months with the formation of a small scar. Infection occurs during bathing by rubbing the skin against the rough cement lining of a swimming pool or aquarium or by touching tropical fish. For treatment, a combination of rifampicin (600 mg/day on an empty stomach) with minocycline or doxycycline (100-200 mg per day) is used, together with clarithromycin (500 mg twice daily), cotrimoxazole (twice 800/160) or ethambutol (max. 2.5 g/day).

The disease must not be confused with Erysipeloid (Rosenbach’s disease), an infection caused by the Gram-positive bacterium Erysipelothrix rhusiopathiae. Infections with this organism also occur frequently in fishermen and people who handle crabs. Pig slaughterers represent another risk group. Cat scratch disease, leishmaniasis and sporotrichosis are to be considered in the differential diagnosis.

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Tropical ulcer

Summary

- Fusospirillary association (*Fusobacterium* + *Borrelia*)
- Initially very painful, subsequently painless ulcer on feet or lower leg
- Bad smell in early stage
- Very chronic course with frequent relapses
- Treatment with antibiotics, local care and skin grafts

Introduction

Tropical ulcer or phagedenic ulcer is a disease of warm and moist geographical regions. There is an association with poor living conditions: lack of clean water, lack of basic health services, carelessness in the treatment of small wounds, abundance of flies, etc. The role of malnutrition and lack of hygiene is clear. For example, in 1942-1945 the disease was extremely common and severe in Western prisoners of war in Japanese camps in Southeast Asia.

In early lesions, Vincent’s fusospirillary bacterial association is usually detected: *Fusobacterium fusiformis* and *Borrelia vincenti*. The same organisms are isolated from the mouth in a third of the patients, from which it is deduced that the cause of tropical ulcer might probably be transmitted to small wounds by saliva. In 1989, two new species of *Fusobacterium* were isolated from tropical ulcers but their exact role in the aetiology has not been determined. In more chronic cases the flora is non-specific. The histological presentation is non-specific. It is possible that tropical ulcer is initially caused by a trivial infection or secondary infection with streptococci or staphylococci in an undernourished person.

Clinical aspects
Tropical ulcers
Tropical ulcers

The primary localizations are on the lower leg, the front of the ankle and the dorsum of the foot. These are sites where the bone lies immediately beneath the skin and where the blood supply is less extensive. In this respect they resemble stasis ulcers in venous insufficiency. In tropical ulcer there are no signs of venous insufficiency. Ulcers occur less often on other parts of the body. Schematically, the disease progresses in three stages:

Acute stage: Local swelling of the skin, oedematous, violently painful and pruritic, sometimes with general symptoms such as fever. A blister with serous or bloody content forms and rapidly bursts. The small ulcer then extends both peripherally and inwards. The patient sometimes reports a recent minor trauma e.g a thorn prick or an insect bite at this site.
Subacute stage: On the ulcer, a superficially necrotic, evil-smelling, purulent, yellow-green or haemorrhagic black coating forms. The base is granular and bleeds easily. Deep in the ulcer the tendons, aponeuroses and periosteum can be seen. The edge of the ulcer is raised but with little if any undermining (in contrast to Buruli ulcers). After a few weeks, the ulcer’s diameter is on average 10-12 cm. The form is or becomes regular, round or oval. Painful lymphadenitis may be present.

Chronic stage: After approximately one month, the swelling and pain decrease. The edge becomes flatter. The base is now coarsely granular, less haemorrhagic and forms less exudate, but the odour persists. Bacteriologically, the flora is now non-specific. Beneath the base of the ulcer there is reactional periostitis in chronic cases. The ulcer gradually heals spontaneously. The longer the disease course, the more difficult healing becomes and the more readily a relapse occurs, as the scar always consists of a small amount of connective tissue lined with fragile, smooth, shiny, often depigmented and atrophic skin. If the lifestyle is not changed, the ulcer flares up again at the first opportunity.

Complications are numerous:

Malformations and functional disorders. Scars with fibrosis of the deeper muscles and stiffness of the ankle joint cause all kinds of problems, of which the most common is retraction of the Achilles tendon with club feet of the equinovarus type.

Secondary infection can lead to tetanus, gas gangrene or cellulitis. Thrombosis of the large arteries can result in distal gangrene. Bleeding can occur as a result of erosion of blood vessels.

Osteomyelitis. There is often a limited cortical reactional osteitis. Extensive destruction of the bone under the ulcer is suggestive of cancer.

Carcinoma. Almost always involves spinocellular epithelioma of the skin with a starting point in the border of the ulcer (“Marjolin’ ulcer”). Cancer occurs after a prolonged course, whether as the gradual degeneration of an active ulcer or in a scar after one or more recurrent episodes of the ulcer. The cancer then develops in the scar itself but also sometimes in the apparently healthy skin. The edges are partially or completely raised. The base is irregular and bleeds readily. There is induration and the ulcer becomes irregular. Spontaneous fractures and spontaneous complete amputation of the lower leg can occur. In 85% of cases the ipsilateral lymph nodes are enlarged, but only a third of these by metastases, the remainder as a result of lymphadenitis. Histological examination provides formal diagnosis. The biopsy site must be carefully chosen as not all the ulcer is necessarily degenerated. Metastases in the lymph nodes can also only be confirmed by biopsy.
**Tropical ulcer, differential diagnosis**

See differential diagnosis ‘Buruli Ulcer’

**Prognosis and social importance**

The importance of this rural disease is usually underestimated. Allowance must be made for the following factors:

- High prevalence, which is rapidly reduced as living conditions are improved: better nutrition, clean water, primary health care services, etc.
- Numerous health centre consultations for tropical ulcer. The disease takes up much of the personnel’s time for treatment, disinfection and bandages.
- Multiple and long-term admissions.
- Frequent relapse.
- Severe invalidity in many patients.
- High incidence of cancer formation, which is a potentially fatal complication. The risk of cancer formation in a poorly treated or untreated tropical ulcer is estimated at 10-15%.

**Treatment**

**Acute cases**

Local and systemic treatment with penicillin is indicated. The results are good if the ulcer is recent and its diameter is less than 2.5 cm. Some tropical ulcers heal in 2-3 weeks after administration of metronidazole for 7 days. Metronidazole is effective against anaerobic organisms.

**Chronic ulcers**

Antibiotics improve the case but do not heal the ulcer. Immobilisation and local treatment e.g. by bathing with Dakin’s solution (aqueous sodium hypochlorite solution) and parenteral antibiotics can result in healing after a few weeks. Effective treatment of a chronic tropical ulcer involves complete excision followed by skin transplants. This can be performed under either general or epidural anaesthesia. The ulcer is curetted until there is diffuse bleeding from the whole underlaying surface. The skin is cut away for up to 0.5 cm at the edges of the ulcer. The underlying bone is vigorously curetted in order to remove sequesters and irregularities and to obtain a flat area. Powder with sulphonamides or antibiotics is then sprinkled on the wound and a pressure bandage applied on top. If
the ulcer is next to a joint, this is immobilised with a plaster of Paris. At the same time antibiotics are administered parenterally. After one week the bandage is removed, the wound cleaned, and skin grafts applied. These are obtained with a dermatome from the heterolateral thigh.

In this way up to 90% of tropical ulcers can heal in less than 3 weeks and leave an acceptable scar.

**Malignant degeneration**

Treatment consists of conservative amputation with adaptation of the stump for a simple prosthesis. The inguinal lymph nodes are removed for histological examination. These tumours metastasise haematogenous and the prognosis is unfavourable.

**Prevention**

Peripheral health centres should provide proper wound care. It is important to promote:

- Decentralisation of primary health care services which can tend small wounds effectively: antiseptics, simple, clean, non-hermetic bandage, penicillin if necessary
- Proper diet with sufficient animal proteins
- Good water supply
- Health education
- Monitoring at the workplace of people with tropical ulcer scars or who suffer a deterioration in their nutritional or health status

**Noma**

Noma (Gr. numein: to devour) or cancrum oris is a terrible gangrenous disease which leads to severe soft and hard tissue destruction in the face (mouth, teeth, lips, nose, cheeks) with lasting disfigurement. It is associated with a high mortality. The exact aetiology is not yet known, but it is thought that several factors contribute to this devastating illness. It is clearly a disease of poverty and social deprivation. Improvements in general socioeconomic status, public health and nutrition made that noma disappeared from all places except the most desperately poor and where severe malnutrition occurs. Several factors contribute, such as malnutrition with associated vitamin and trace element deficiencies, poor oral hygiene, a compromised immune status (malnutrition, measles, CMV
infection, blood dyscrasia such as leukaemia), a lesion of the gingival mucosal barrier, a (bacterial?) trigger and inappropriate initial treatment. They probably act together to cause noma. Bacteria such as spirochaetes, *Prevotella intermedia* and *Fusobacterium necrophorans* are suspected to play a role in the acute pathology. However, it should be remembered that at present, most bacteria in the mouth cannot be cultured in vitro. Although the disease existed in Europe and other parts of the globe, at present it is most common in Africa. The disease affects mostly children between 2-6 years but can occasionally appear in older children and even in debilitated adults (Auschwitz!). It is thought that the disease starts as an acute painful necrotising gingivitis (“trench mouth”), evolving to a necrotising stomatitis with ulceration and oedema of the cheek. The lesion tends to start at the alveolar margin in the premolar-molar region. It spreads very fast (1-2 days). Within a couple of days, a greyish area appears on the cheek. This becomes black and necrotic and has well defined margins. There is an offensive odour. The necrotic zone penetrates the cheek and has a typical cone shape (“cône gangrénéux”). After the necrotic tissue has sloughed away, bone is exposed. Large bone sequesters may form, sometimes with destruction of maxilla and/or mandibule. It should be distinguished from pyogenic abscesses and Burkitt’s lymphoma. Secondary infection occurs rapidly, as can be expected. Fever occurs in some patients. Many patients die due to starvation, septicaemia, or aspiration pneumonia. Because of the high mortality in acute noma and the fact that it occurs in the poorest among us in areas with inadequate reporting, the burden of disease is difficult to determine for epidemiologists.
Noma, cancrum oris. Photo Cochabamba, Bolivia
Noma, cancrum oris. Face ulcer. Photo Cochabamba

The tissue defects are classified in 4 types:

- Type I is the most common and consists of a localised cheek and commissural defect. It can be bilateral.
- Type II includes the upper lip, and in some cases the nose and the palate.
- Type III is located on the lower lip ± the mandible and floor of the mouth.
- Type IV consists of major defects of the whole cheek, lips, palate, maxilla and can extend up to the orbit, eyelids and nose.

Treatment in the acute phase encompasses proper oral hygiene, mouth rinses with chlorhexidine, antibiotics including penicillin and metronidazole against anaerobic bacteria, proper nutrition and vitamin/trace element supplements and treatment of any underlying medical conditions. The healing is characterized by ugly scars with fibrous tissue which tends to provoke strictures. After the acute phase, physiotherapy should be initiated to limit the strictures, fibrous scarring, trismus and to avoid bony ankylosis (bridging) between upper and lower jaw. Bundles of wooden spatulae in the mouth or more sophisticated devices (e.g. the Therabite) are used. At least a year after the initial disease, reconstructive craniomaxillofacial surgery for the sequelae can be considered. This should be done by experienced teams including specialised surgeons and anaesthesiologists (tracheostomies, fiberoptic intranasal intubation). Each case will require an individual approach.
Keloids on the face, shaven area (microtraumata). Copyright ITM
Keloid of the ears, as reaction to perforations for aesthetic reasons. Copyright ITM

Keloids are nodular, often lobulated, firm to hard but movable, non-encapsulated masses of hyperplastic scar tissue. It is a result of an overgrowth of granulation tissue (collagen type 3, early) at the site of a healed skin injury which is then slowly replaced by collagen type 1 (late). The pathogenesis is complex and involves both genetic and environmental factors and the exact mechanism is still unknown. Growth factors like VEGF, TGF-β1, TGF, β2, CTGF and PDGF-α play are overexpressed, but it remains unclear if this is the cause or the consequence of the excessive scarring. Keloids can closely resemble lobomycosis but can also be confused with lepromata and less likely with lesions of diffuse cutaneous leishmaniasis. Africans are particularly susceptible to keloids. The tribal scar pattern following scarification is based on this property. Keloids occur in all types of conditions, for example after burns, cauterisation, vaccinations, on in-growing beard hair, folliculitis or even spontaneously. Keloids are raised and sharply delineated. The overlying skin is reddish and shiny. The lesion can be itchy or painless and the dimensions can be unexpectedly large. Keloids can develop later, up to years after the initial trauma. Treatment is difficult. Treatment options include resection, cryotherapy, intralaesional corticosteroids, 5-fluorouracil or bleomycin. Complete excision is followed by recurrence in 70% of cases. Excision within the edges of the lesion is recommended but the result is aesthetically unsatisfactory. Corticosteroids have no effect on the fixed lesions, but can prevent their recurrence by injections localised around the site of the original lesion if started 3 weeks after surgery and repeated weekly for the following 8-12 weeks. Bigger and horizontally growing keloids are more likely to recur after treatment.

Diphtheria

Summary

- Caused by the gram-positive bacillus Corynebacterium diphtheriae
- Infection leads to respiratory or cutaneous disease or an asymptomatic carrier state
- The pseudomembranes in combination with neck swelling can cause life threatening croup
- Diagnosis in most low-resource settings is clinical
- Treatment with erythromycin or penicillin
- Antitoxin and airway protection in severe cases
- Worldwide vaccination lead to a significant decrease in diphtheria cases
General

Diphtheria is an infectious disease caused by the gram-positive bacillus Corynebacterium diphtheriae. Symptoms range from mild to severe. Whereas in the 1980s about 100,000 cases were reported worldwide, in 2015 this number had dropped to 4,500 cases with >80% vaccination rates worldwide. Regions mostly affected are sub-Saharan Africa, the Indian subcontinent and Indonesia where mostly children are affected. In 2015, 2,100 deaths were reported, down from 8,000 in 1990. The disease has become rare in high-income countries thanks to widespread vaccination but re-emergence is a threat when vaccination rates decrease. Diphtheria death rate in those diagnosed varies from 5% to 10%.

There are four types of C. diphtheria: gravis, intermedius, mitis and belfanti. All four can cause Diphtheria, although mitis strains cause less severe disease. Symptoms are caused by bacterium’s toxin. In rare occasions toxigenic strains of other Corynebacterium species (C. ulcerans, C. haemolyticum, C. pseudotuberculosis) evoke respiratory symptoms.

The name comes from the Greek word “diphthera” which means “leather” referring to the appearance of the pseudomembrane in the throat.


The disease was first described in the 5th century BC by Hippocrates. In 1613, Spain experienced an epidemic of diphtheria. The year is known as El Año de los Garrotillos (The Year of Strangulations) in the history of Spain. Before 1826, diphtheria was known by different names across the world. In England, it was known as Boulogne sore throat, as it spread from France. In 1826, Pierre Bretonneau gave the disease the name diphthérite (from Greek diphthera “leather”) describing the appearance of pseudomembrane in the throat.

In 1878, Queen Victoria’s daughter Princess Alice and her family became infected with diphtheria, causing two deaths, Princess Marie of Hesse and by Rhine and Princess Alice herself. In 1883, Edwin Klebs identified the bacterium causing diphtheria and named it Klebs-Loeffler bacterium. The club shape of this bacterium helped Edwin to differentiate it from other bacteria. Over the period of time, it was called Microsporon diphtheriticum, Bacillus diphtheriae, and Mycobacterium diphtheriae. Current nomenclature is Corynebacterium diphtheriae.
Friedrich Loeffler was the first person to cultivate *C. diphtheriae* in 1884. He used Koch’s postulates to prove association between *C. diphtheriae* and diphtheria. He also showed that the bacillus produces an exotoxin.

Joseph P. O’Dwyer introduced the O’Dwyer tube for laryngeal intubation in patients with an obstructed larynx in 1885. It soon replaced tracheostomy as the emergency diphtheric intubation method.

In 1888, Emile Roux and Alexandre Yersin showed that a substance produced by *C. diphtheriae* caused symptoms of diphtheria in animals.

In 1890, Shibasaburo Kitasato and Emil von Behring immunized guinea pigs with heat-treated diphtheria toxin. They also immunized goats and horses in the same way and showed that an “antitoxin” made from serum of immunized animals could cure the disease in non-immunized animals. Behring used this antitoxin (now known to consist of antibodies that neutralize the toxin produced by *C. diphtheriae*) for human trials in 1891, but they were unsuccessful. Successful treatment of human patients with horse-derived antitoxin began in 1894, after production and quantification of antitoxin had been optimized. Von Behring won the first Nobel Prize in medicine in 1901 for his work on diphtheria.


In 1897, Paul Ehrlich developed a standardized unit of measure for diphtheria antitoxin. This was the first ever standardization of a biological product, and played an important role in future developmental work on sera and vaccines.

In 1901, 10 of 11 inoculated St. Louis children died from contaminated diphtheria antitoxin. The horse from which the antitoxin was derived died of tetanus. This incident, coupled with a tetanus outbreak in Camden, New Jersey, played an important part in initiating federal regulation of biologic products.

In the 1920s, each year an estimated 100,000 to 200,000 diphtheria cases and 13,000 to 15,000 deaths occurred in the United States. Children represented a large majority of these cases and fatalities. One of the most infamous outbreaks of diphtheria was in Nome, Alaska; the “Great Race of Mercy” to deliver diphtheria antitoxin is now celebrated by the Iditarod Trail Sled Dog Race.

In 1926, Alexander Thomas Glenny increased the effectiveness of diphtheria toxoid (a modified version of the toxin used for vaccination) by treating it with aluminium salts. Vaccination with toxoid was not widely used until the early 1930s.

In 1943, diphtheria outbreaks accompanied war and disruption in Europe. The 1 million cases in Europe resulted in 50,000 deaths.

In 1974, the World Health Organization included DPT vaccine in their Expanded Programme on Immunization for developing countries.
Transmission

Diphtheria is airborne and spreads between people by coughing and sneezing. In rare occasions, direct contact with diphtheria skin lesions can transmit the bacteria. Indirect transmission is possible when an infected person touches an object on which the bacteria can remain viable. Asymptomatic carriers exist and they can still spread the infection to others. Immunity from past infection or vaccination does not prevent carriage of the bacterium.

Diphtheria toxin

*C. diphtheria* produces and exotoxin when it is infected with a bacteriophage that integrates the toxin-encoding gene (tox+) into the bacteria. Diphtheria toxin is composed of two peptide chains: fragment A and fragment B. Fragment B facilitates toxin entry into host cells by binding the heparin-binding EGF-like growth factor on the cell membrane. Once inside the cell’s endosome, a trypsin-like protease splits the toxin in the A and B fragments. The low pH in the endosome causes fragment B to create pores in the endosome membrane through which fragment A can enter the cytoplasm. Fragment A catalyses ADP-ribosylation of elongation factor EF-2, a protein that moves tRNA from the A-site to the P-site of the ribosome during the translation step in protein synthesis. The final result is a disturbed protein synthesis leading to cell death.

Clinical aspects

The incubation period is usually two to five days and the disease starts with a gradual onset of sore throat with pharyngeal erythema and fever. In more severe cases diphtheria destroys the respiratory tract tissues with dead tissues forming a thick, grey, friable and tightly adhering coating in the throat. This is called a pseudomembrane which is composed of necrotic fibrin, white- and red blood cells, epithelial cells and bacteria. The pseudomembrane may expand from the nose to the tonsils, the throat up to the bronchial tree. This can lead to dysphagia and can obstruct the airways provoking hoarseness, stridor and sometimes suffocation when membranes are aspirated. This can be exacerbated with extreme neck swelling (“bull neck”) due to enlarged lymph nodes causing external pressure on the airways. This clinical picture is referred to as “diphtheritic croup” or “true croup” (= laryngotracheobronchitis caused by diphtheria). Children who have smaller airways are more vulnerable to the complications of diphtheritic croup. Nowadays, croup is mostly related to viral infections causing milder respiratory symptoms.
Diphtheria can be complicated by myocarditis (in two-thirds of severe cases) and nerve inflammation (in up to 75 percent of severe diphtheria). Paralysis of the soft palate and posterior pharyngeal wall can occur, as well as cranial nerve paralysis. Cardiac and neurological symptoms often arise from the moment respiratory symptoms are improving. A peripheral polyneuropathy can develop weeks or months after the acute illness.

Cutaneous diphtheria presents as chronic, non-healing ulcers with a grey membrane. The infection is often preceded by local trauma. Epidemics of cutaneous diphtheria have occurred in populations living in poor hygienic conditions. The ulcers can serve as a reservoir from which the infection spreads to others.

**Diagnosis**

Diagnosis is often made considering the setting and clinical manifestations in a non-vaccinated person. Positive cultures confirm the diagnosis, but the need for special culture media (Löffler’s or Tindale’s media), the need for appropriate transport media and the necessity of quick inoculation, make the confirmation challenging, even in high-resource settings. Toxin detection with PCR is possible and confirms that the strain is toxigenic.

A probable case is a clinically compatible case that is not laboratory-confirmed nor epidemiologically linked to a confirmed case. A confirmed case is a clinically compatible case that is laboratory confirmed or epidemiologically linked to a laboratory-confirmed case. It is important that the antitoxin and antibiotics are administered prior to confirmation when diphtheritic croup is suspected. Cases of diphtheria should be reported to the World Health Organization (WHO).

**Differential diagnosis**

Several diseases can give a clinical picture that can resemble pharyngitis with pseudomembranes: infectious mononucleosis, group A streptococcal tonsillopharyngitis, epiglottitis, viral pharyngitis, Vincent’s angina (= acute necrotizing ulcerative gingivitis), oral candidiasis, pertussis (100-day cough).

**Treatment**

When diphtheria is suspected, prompt initiation of antibiotics is needed since severe untreated diphtheria has a mortality rate of 40% to 50%. Erythromycin (500 mg 4 times daily, 14 days) and penicillin G (300,000 IU IM daily for patients < 10 kg and 600,000 IU IM daily for patients > 10 kg) followed by penicillin V (250 mg 4 times daily, oral) for a total of 14 days are the antibiotics of choice.
In severe diphtheria with pseudomembranes or cardiac involvement, diphtheria antitoxin is indicated. These antibodies are produced in horses that have been challenged with diphtheria toxin. The antitoxin does not neutralize toxin that is already bound to tissues, hence a delay in administration increases mortality rates. In about 10 percent of patients receiving antitoxin hypersensitivity or serum sickness arises.

In case of (threatening) respiratory failure airway protection with intubation is necessary. This procedure can be difficult if there is extensive throat oedema and mucosal friability. There is a risk of dislodging the pseudomembranes into the bronchi. In rare occasions a tracheotomy is needed. After recovery, vaccination is still needed since pharyngeal infections do not protect against future infections. Skin infections are an exception since they evoke a strong antibody response.

**Prevention**

An effective vaccine exists with different available formulations. In childhood, three or four doses are given along with tetanus and pertussis in a penta-, sexta- or heptavalent vaccine. A booster vaccination, together with tetanus, is recommended every ten years.

Close contacts can be given prophylaxis with a single dose of penicillin G benzathine (1.200.000 IU IM) or oral erythromycin 500 mg 4 times daily for 1 week.

**Tetanus**

**Summary**

- Tetanus: symptoms caused by a powerful toxin from anaerobic bacteria
- Pathogenic organism present in wounds, umbilical stump infections
- Prevention by vaccination including pregnant women
- Clinical diagnosis
- Painful muscle spasms (spontaneous and after provocation), normal consciousness
- Treatment: wound care, antitoxin, anti-spasmodics, clear airways, supportive measures
- Avoid or treat complications
General

Tetanus is a disease caused by the toxin produced by an anaerobic bacterium: *Clostridium tetani*. This disease is completely preventable by vaccination therefore it is particularly tragic that it still occurs. The disease cannot be transmitted from human-to-human. *Clostridium tetani* is a strictly anaerobic Gram-positive rod-shaped bacterium, in cultures or in tissue it can be Gram-variable. It forms a characteristic spore at one end (exclamation mark, tennis racket). These spores are very resistant: they resist boiling, short autoclaving, alcohol and phenol. They are destroyed by autoclaving at 121°C for at least 12 minutes (better 15′). The bacterium occurs widely in nature for example in the soil and in the intestinal tract (especially of cattle and horses). Approximately 10% of people have C. tetani in their colon.

Neurotoxin

If the organism infects a wound where the oxygen concentration is low (interrupted vascularization, foreign body, tissue necrosis, umbilical stump) the bacterium can multiply. The bacterium itself is not invasive. The pathogenic organism produces a neurotoxin- tetanospasmin. This is released when the organism lyases. This protein is responsible for all the clinical manifestations of tetanus. The toxin is cleaved outside the cell by a bacterial protease into a heavy and a light chain. The toxin enters the neuromuscular junction. Once internalized, it migrates via the fast retrograde axonal transport pathway of the peripheral nerves towards the nerve soma located in the spinal cord. Another pathway which is hypothesised is via the lymphatics and the blood to the central nervous system. The neurotoxin inside the motor neurons translocates (crosses the synapse) to inhibitory interneurons. There the toxin cleaves the protein synaptobrevin which is present on the presynaptic vesicles which contain the inhibiting neurotransmitters GABA and glycine. Due to the removal of synaptobrevin on the exterior of the vesicles the latter can no longer fuse with the synaptic membrane. Therefore the reflex arc cannot be inhibited. The consequence of the removal of the normal inhibition of the motor neurones is increased muscle tone at rest and tonic spasms. The toxin is also active on the sympathetic nervous system. The role of a second toxin- tetanolysin is still unclear.

GABA (gamma- Aminobutyric Acid)

Throughout the central nervous system, GABA is an inhibitory neurotransmitter. GABA receptors open channels for negatively charged chloride ions, hyperpolarizing the neuronal membrane and making it less likely that action potentials can be generated in output neurons.

Tetanospasmin is one of the most powerful toxins known to man (botulinum toxin is the undisputed
leader). The toxin is present in the body at such low doses that it does not trigger an immunological response. **Tetanus can therefore be contracted more than once.** That is one reason why people with clinical tetanus should still be vaccinated.

Most cases of tetanus occur after wounds (lacerations, bites, burns, pricks, IM injections, umbilical infections in neonates, infected abortions, a sand flea burrowing under a toenail, infected Guinea worm). Sometimes the focus is a middle-ear infection (otitis media with perforated ear drum). In 20 to 30% of tetanus patients no entry point or wound can be found.

## Clinical aspects

[Photo: Neonatal tetanus with opisthotonus. Photo Cochabamba, Bolivia]
Tetanus, adult man. Notice the slight opisthotonus. Copyright ITM

The incubation period varies: the shorter it is the more serious the infection. Neonates who contract tetanus before they are 7 days old almost never survive. The incubation period varies between a few days and a few weeks. Three clinical forms can be distinguished:

Localized tetanus: rigidity and painful spasms in a group of muscles in the area of the wound, without general involvement. This form is rare. It is sometimes prolonged for months. The mortality rate is < 1%.

Generalized tetanus, including neonatal tetanus: first there is a short period of restlessness, irritability, dysphagia and sweating. Trismus frequently occurs (spasms of the masseter = jaw muscle). Patients are no longer able to open their mouths wide. Another name for tetanus is “lockjaw”, which refers to the trismus. If the spasms spread to the other muscles of the face a spastic grimace sets in: risus sardonicus (“bitter laugh”). The disease typically descends; after the jaws and
the face to follow the neck, back, abdomen and finally the extremities. Back muscle spasms lead to arching backwards (opisthotonus). Successive attacks of opisthotonus are characteristic. The spasms are very painful and last a few seconds to a few minutes. They can occur spontaneously or are elicited by all kinds of stimuli (sudden noises, touching, sudden bright light). Because the latter is a well-known phenomenon, the patients are sometimes placed in a dark room. This sometimes leads to insufficient nursing care with serious consequences. The body temperature, heart rate and blood pressure are variable because the autonomic nervous system is also affected. In most cases there is rather low to moderate fever but hyperpyrexic periods do occur.

**Cephalic form:** Occasionally a true cephalic form occurs, with symptoms affecting the head, throat and neck; while sparing the rest of the body.

**Differential diagnosis:**

- Generalized tetanus
- Bacterial meningitis and subarachnoidal haemorrhage: lumbar puncture
- Epilepsy: no muscle rigidity between spasms, history of previous episodes
- Extrapyramidal reactions and dystonias while on neuroleptics, such as phenothiazines e.g. chlorpromazine (Largactil®) or metoclopramide (Primperan®).
- Cerebral malaria: thick film test, no muscle rigidity between convulsions

Acute strychnine poisoning resembles tetanus very closely, and an old proposed name for strychnine was “tetanine”. This bitter colourless alkaloid is obtained from the ripe seeds of *Strychnos nuxvomica* and related plants, such as Saint Ignatius beans (*Strychnos ignatia*) and snake wood (*Strychnos colubrina*). The plant seeds are sometimes used in traditional medicine (e.g. in Cambodia). It is a competitive antagonist of glycine, an inhibitory neurotransmitter. There are face spasms followed by hyperreflexia in the legs and arms. This is followed, a little later by painful generalised convulsions, triggered by sudden sounds or stimuli. The patient may be conscious. Finally breathing difficulties and coma follow. Upon death, rigor mortis sets in very quickly. If the patient survives recovery is fairly quick unlike tetanus.

Hypocalcaemic tetany after accidental parathyroidectomy or in primary hypoparathyroidia is rare. The parathyroid glands secrete parathyroid hormone which increases the concentration of calcium in the
blood. If there is a shortage of parathyroid hormone, the calcium levels in the blood fall and convulsions may occur. There may be spasms of the hands and feet as well as tingling around the mouth. Trismus is rare. Chvostek’s and Trousseau’s sign may be present.

Rabies: hydrophobia, periods of confusion, brain stem symptoms and cranial nerves being affected.

Trismus

- Dental abscess, peritonsillar abscess
- Pharyngeal diphtheria
- Fracture of the mandible
- Mumps

**Diagnosis**

The diagnosis is purely clinical. Repeated tonic spasms with muscle rigidity between the convulsions are typical. Spasms can be triggered by sudden stimuli: e.g. clapping the hands. The patient is fully conscious. *Clostridium tetani* can be found in wounds in less than 30% of cases, but a microbiological diagnosis via culture is less important than making a clinical diagnosis. The cerebrospinal fluid is normal.

**Treatment**

Tetanus is a disease which can drag on for weeks. There is high mortality. Treatment consists mainly of neutralising toxin and preventing convulsions and complications. Thorough cleansing of the wound and good nursing care are the most important factors in determining whether the patient survives or not.

1. The pathogenic organism, *Clostridium tetani*, has to be eradicated: by wound cleansing (hydrogen peroxide, povidone iodine [Iso-Betadine®], debridement) and penicillin G preferably IV, e.g. 1 to 12 million units per day. However, it is possible that penicillin G may act on GABA transmission and exacerbate the toxin’s effect. Therefore, the use of penicillin is controversial. Metronidazole is sometimes recommended instead.

2. The toxin which is still circulating must be neutralised with antitoxin. Human hyperimmunoglobulin is best: one single IM injection of 3000 to 6000 IU in two different sites (or 10,000 to 50,000 IU hyperimmune horse serum). Sometimes lower quantities are recommended. Human antiserum has a half-life of 25-28 days therefore it must not be given repeatedly. The half-life of horse antiserum
is somewhat less than 2 weeks. Toxin which has already bound to nerve cells, cannot be removed and is responsible for the repeated spasms. Some guidelines use tetanus immunoglobulins intrathecally.

3. The infection does not produce any immunity so that the patient must also be vaccinated. The vaccine must not be mixed with gammaglobulins and must be injected at another site.

4. Prevention of muscle spasms is important because the spasms are very painful, and they interfere with breathing. They can lead to gastric reflux with aspiration pneumonia. The repeated violent convulsions can even result in patients breaking their own bones. Diazepam (Valium®) is better than barbiturates and is often used as the drug of first choice. Sometimes very large quantities have to be given (50 to 500 mg/day). Respiratory depression can occur. Midazolam (Dormicum®) is an alternative. In the case of depression of the central nervous system, flumazenil (Anexate®) can be used as an antidote. Dantrolene (Dantrium®) can be used but it is very expensive. Chlorpromazine (Largactil®) is also useful. Baclofen (Lioresal®) is a GABA B receptor agonist that inhibits pre-synaptic acetylcholine release and synaptic medullar reflexes (i.e., lowers excitability of motor neurons), which results in an antispastic action. It is rarely available in low resource setting. If possible, baclofen can also be administered intrathecally.

5. Trismus, dysphagia, laryngeal spasms, respiratory muscle spasms, gastric reflux and sedatives can lead to pulmonary complications. Aspiration of secretions to clear the airway is necessary. Oxygen will often be given. Sometimes tracheostomy (severe laryngeal spasms) is performed. The indications for tracheostomy are acute airway obstruction due to laryngeal spasms that interfere with respiration, or to facilitate mechanical ventilation. If the means are available, curare (muscle relaxant, e.g. pancuronium = Pavulon®, vecuronium) and mechanical ventilation can be used.

6. The use of magnesium sulfate infusions in the management of tetanus enables one to minimize sedation and reduce the need for mechanical ventilation, and thereby greatly simplifying the care of the tetanus patient. Magnesium is also able to minimize sympathetic overactivity associated with tetanus. Furthermore, magnesium sulfate is already a well-known entity due to its extensive use in the management of pregnancy induced hypertension. As a guide line for an adult, a loading dose of 5 gram is given, followed by 2-3 gram per hour afterwards.

7. The patient must be regularly turned to prevent pressure sores. The risk of pulmonary embolism decreases with subcutaneous heparinisation. Low-molecular heparin can be given prophylactically, but this is often not available in the tropics. Feeding is performed mainly via a thin flexible nasogastric tube (the patient cannot eat for weeks), his is sometimes overlooked. Urinary catheterisation is necessary to prevent urine retention.

8. Septicaemia occurs frequently in neonatal tetanus (umbilical stump as the point of entry) and must not be ignored. In third world countries, it is not unusual for the umbilical stump to be covered with various contaminated herbs, animal droppings or fats.

9. Beta-blockers such as labetalol can be administered in cases of excessive sympathetic tone. In the
case of hypotension, IV fluid and vasopressors should be administered if available.

**Example of “Adult tetanus protocol”**

1. Start metronidazole intravenously 500mg three times a day.
2. Give tetanus human immune globulin IM 3,000-6,000 iu if available. If not available Equine ATS 10,000 iu IM.
3. Admit ICU, commence oxygen, IV access and monitoring.
4. Alert surgeon to do radical debridement. Nasogastric tube may be passed during surgery.
5. Slow loading dose diazepam IV to control spasms. Up to about 40mg may be required. Give a loading dose of 5g magnesium sulphate slowly over 20 minutes IV.
6. Start diazepam 10mg 6 hourly and increase to hourly if required. Titrate to symptoms.
7. Start magnesium 2.5g IV 2 hourly and increase to hourly if required. Titrate to symptoms. Stop diazepam if symptoms controlled by magnesium alone.
8. Phenobarbitone up to 200mg IV twice a day for breakthrough spasms using 50mg doses.
10. Intermittent positive pressure ventilation with muscle relaxants if respiration compromised by treatment or uncontrolled spasms.

**Prognosis**

**Incubation period < 7 days:**

1. The course of the disease is always very serious.
2. The interval between the first symptoms and generalized spasms is 3 days or less.
3. Mortality rate > 80 %

**Incubation 7 to 10 days:**

1. Moderately severe course with the symptoms developing over 3 to 6 days.
2. The mortality rate varies from centre to centre.

**Incubation > 10 days:**

1. Milder course with the usual symptoms setting in slowly.
2. Generalized convulsions are sometimes absent.
3. If a baby survives neonatal tetanus there is an increased risk of permanent brain damage, with
behavioural and developmental problems as well as difficulties with fine motor movements.

**Prevention**

In the case of a wound which is likely infected with C. tetani, prior to symptoms development; in addition to wound care and tetanus vaccination, human hyperimmunoglobulins are given intramuscularly, i.e. 250 to 500 IU once only. Hyperimmune horse serum can be used but this sometimes leads to anaphylactic reactions and serum sickness. Tetanus toxoid (toxin inactivated by formalin) is used for vaccination. The vaccine is administered intramuscularly on 3 occasions with a minimum interval of one month between each injection. There is a booster after 1 year and then every 10 years (or after 5 years if injured). It is best if children are vaccinated at the age of 2, 4, 6 and 15 months of age. This series is completed with a dose between 4 and 6 years. Additional boosters are given every 10 years after that. A serum antitoxin concentration of 0.01 IU/ml is regarded as protecting against tetanus. This determination can only be carried out in a few laboratories.

The antibodies (particularly subclass IgG1) cross the placenta from mother to child and protect the neonate from neonatal tetanus. These antibodies gradually disappear from the child’s blood over the following months. Vaccination of the mother is therefore part of the prenatal consultation. The vaccine is very efficient and very safe. It is part of the EPI (extended programme of immunization) of the WHO.

**Regionally relevent pathogens**

**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 Gram-positive 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, differential diagnosis**

**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

**Cutaneous anthrax, diagnosis**

A **Gram stain of a smear of the lesion** shows the typical large Gram-positive rods (1-1.5 \( \times \) 4-10 \( \mu \)m). 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.
**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 tularemia (*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 cefazidime 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 basin, a shallow eastern basin 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 biowarfare 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.

**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 man, 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. Raccoons, 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 axillary 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 aspecific. **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, pneumatic 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 erlichioses. 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).

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.
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).

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!).
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°C lower than that of non-infected fleas. Healthy rats have a body temperature of ± 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-to-human 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 fermentation 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.
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, tularemia
• 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 (ciprofloxacin 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, flea 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 fluoracetate 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.

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, herdsman, 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 sacroiliac 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 non-granulomatous form. Fresh active lesions of the choroid and retina appear as yellowish-white 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 toxocariasis 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).

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).


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 micro-abscesses?). 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
Bacteria | 126

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.

Melioidosis 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 bloodstream 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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Rickettsiosis and related infections

LAST UPDATED BY ADMIN ON DECEMBER 16TH, 2022

Rickettsiosis

Summary

- Rickettsioses: bacterial infections of varying degrees of severity
- Transmission of typhus via lice, flies, ticks or mites
- Basic lesion is vasculitic
- Fever, rash, sometimes chancre and multi-organ involvement
- Hepatosplenomegaly, neurological signs, heart failure, renal insufficiency, bleeding
- Diagnosis clinical and often difficult; serological tests and PCR often not available
- Treatment with tetracyclines (1st choice)
General

Rickettsiae are very small bacteria (0.8 x 0.4 µm) that belong to the alpha-group of purple bacteria. The Rickettsiacea family contains the genera *Rickettsia* and *Orienta*. These coccobacilli are closely related to *Bartonella*, *Wolbachia*, *Cowdria* and *Anaplasma*. They multiply intracellularly. They have a Gram-negative cell wall structure, but cannot be detected by Gram staining, although they can be by Giemsa staining – with difficulty.

**Rickettsia discovery**

They bacterium derives its name from the American researcher, Howard Ricketts, who discovered them in 1909 in Montana, USA, as the source of a serious disease (Rocky Mountain Spotted Fever = RMSF caused by *Rickettsia rickettsiae*). Originally the disease was called Black Measles due to the spotted rash throughout the body of infected patients. Howard himself died from typhus in an epidemic in Mexico some years later. In 1916, Henrique da Roche Lima discovered *Rickettsia prowazekii*, the bacterium that causes epidemic typhus. He named it after his colleague Stanislaus van Prowazek, who had died from typhus whilst investigating the diseases in a prison hospital in Hamburg.

**The historical role of Typhus in various armed conflicts**

The Grande Armée of Napoleon Bonaparte lost many soldiers from epidemic typhus during the invasion of Russia in 1812. Of the invading 422,000 soldiers of the Grande Armée, only a few ten thousand (numbers vary according to source) would return due to decimation by epidemic typhus, extreme cold, hunger and to a lesser degree battle. Several decades later during the Crimean War (1854-56) between Russia and England and France on the other, typhus took a high toll. Florence Nightingale was famous for her help to the wounded during this dreadful conflict. In the 1915 Serbian epidemic, it is estimated that nearly all the country’s 400 doctors contracted epidemic typhus and more than a 100 of them died. The scale of the massive epidemics in Eastern Europe and Russia between 1918 and 1922 can hardly be imagined, with an estimated 20-30 million cases and at least 3 million deaths. Now there are occasional flare-ups of epidemic typhus, as in 1997 in Burundi with an estimated 24,000 cases in the first half of that year.
Classifications

Different classifications may be found in many textbooks and manuals, e.g. the “Spotted Fever” group (transmitted by ticks), the typhus group (transmitted by fleas and lice, no outer membrane protein OmpA) and scrub typhus. The division is based on intracellular growth characteristics and on antigenic differences between the various micro-organisms. Organisms of the spotted fever group cause rapid cell lysis and spread rapidly from cell to cell, while *R. prowazekii* - belonging to the typhus group - grows to enormous numbers intracellularly before causing the host cell to burst. Spotted fever group *Rickettsiae* are found in both the nucleus and the cytoplasm, whereas *R. prowazekii* is found in the cytoplasm only. In practical terms these divisions are not useful. They can give rise to confusion rather than clarification.

New Rickettsiae and various subtypes are still regularly being discovered. It is easier just to state that there are various sorts of *Rickettsiae* and that they cause a range of diseases of varying severity. Furthermore, not all Rickettsiae occur everywhere. Thus RMSF is not found in Asia, nor does scrub typhus exist in America.

Another way to classify rickettsioses is according to the transmitting vector, but the patient is often unaware of the ectoparasite that bit him. It is probably more useful to classify Rickettsioses according to their clinical picture’s severity:

### Mainly very serious course

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
<th>Vector</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. prowazekii</em></td>
<td>Epidemic typhus</td>
<td>Louse</td>
<td>Worldwide</td>
</tr>
<tr>
<td><em>R. rickettsii</em></td>
<td>Rocky Mountain SF</td>
<td>Tick</td>
<td>America</td>
</tr>
<tr>
<td><em>O. tsutsugamushi</em></td>
<td>Scrub typhus</td>
<td>Mite</td>
<td>SE-Asia, Australasia</td>
</tr>
</tbody>
</table>

### Mainly mild to moderately severe course

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
<th>Vector</th>
<th>Distribution</th>
</tr>
</thead>
</table>


### Transmission

With the exception of epidemic typhus, rickettsiosis are **zoonoses**. Transmission to humans occurs via **arthropods**. Ticks and mites infect humans through their bite. Lice and fleas infect humans through their faeces. Louse faeces can remain contagious for months. Ticks and mites transmit the organisms to their progeny (transovarial transmission). Mites and ticks are thus both vector and reservoir. In mites, infection with *Orientia tsutsugamushi* causes a shift in the sex-ratio in the offspring of the mites so that the female mites predominate in the following generation. This can be prevented by treating mites with tetracyclines.

#### Typhus transmission via lice

In 1906 Charles Nicolle demonstrated that **infection can be transmitted by body lice** (head lice and public lice are not known to transmit pathogens). Afterwards it was shown that **louse faeces** were infectious. Transmission is also possible when dry louse faeces are **inhaled** via aerosol.

<table>
<thead>
<tr>
<th>Rickettsial Species</th>
<th>Disease Name</th>
<th>Vector</th>
<th>Geographical Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. typhi</em> (<em>mooseri</em>)</td>
<td>Endemic typhus</td>
<td>flea</td>
<td>Worldwide</td>
</tr>
<tr>
<td><em>R. felis</em></td>
<td>Flea typhus</td>
<td>flea</td>
<td>Europe, Americas, Africa, Thailand, New Zealand</td>
</tr>
<tr>
<td><em>R. conorii</em></td>
<td>Fièvre boutonneuse</td>
<td>tick</td>
<td>Mediterranean, Africa (India?)</td>
</tr>
<tr>
<td><em>R. africae</em></td>
<td>African Spotted Fever (SF)</td>
<td>tick</td>
<td>Africa, Caribbean</td>
</tr>
<tr>
<td><em>R. sharoni</em></td>
<td>Israeli SF</td>
<td>tick</td>
<td>Middle East</td>
</tr>
<tr>
<td><em>R. sibirica</em></td>
<td>North Asian SF</td>
<td>tick</td>
<td>Siberia, Mongolia</td>
</tr>
<tr>
<td><em>R. japonica</em></td>
<td>Japanese SF</td>
<td>tick</td>
<td>Japan</td>
</tr>
<tr>
<td><em>R. australis</em></td>
<td>Queensland SF</td>
<td>tick</td>
<td>Australia</td>
</tr>
<tr>
<td><em>R. honei</em></td>
<td>Flinders Island SF</td>
<td>tick</td>
<td>Australia</td>
</tr>
<tr>
<td><em>R. mongolotimonae</em></td>
<td>Atypical fièvre boutonneuse</td>
<td>tick</td>
<td>Asia, Europe, Africa</td>
</tr>
<tr>
<td><em>R. helvetica</em></td>
<td>Influenza syndrome</td>
<td>tick</td>
<td>Europe</td>
</tr>
<tr>
<td><em>R. slovaca</em></td>
<td>Tick-borne lymphadenopathy</td>
<td>tick</td>
<td>Europe</td>
</tr>
<tr>
<td><em>R. akari</em></td>
<td>Rickettsialpox</td>
<td>mite</td>
<td>USA, Africa</td>
</tr>
</tbody>
</table>
Charles Nicolle

At the time Charles Nicolle was working at the Pasteur Institute in Tunis. There were numerous cases of typhus and the hospitals were over-full. In 1909 he observed that personnel in the laundry became infected when they had washed the clothing of people who had been admitted. There was however no secondary infections originating in the over-full hospital wards. Hospitalised patients were given a hot bath with soap and clean hospital clothing on their admission. Dr Nicolle suspected a pathogenic agent in the patients’ dirty clothing and underwear. He injected a chimpanzee with a patient’s blood. After a few days he collected some lice from the animal and introduced these insects into another, non-injected healthy chimpanzee. This second animal in turn became ill after ten days. Control experiments confirmed the results.

People who have previously survived epidemic typhus (R. prowazekii) often harbour the bacteria in their body for life, even though they are asymptomatic (chronic carriers). In the event of immunosuppression, this can result in a mild flare-up of the infection, even after many years (Brill-Zinsser disease). When such a person is in the “right” circumstances, this can cause epidemic louse-borne typhus. As transmission of epidemic typhus occurs through lice, epidemics occur in conditions of poverty, overpopulation and poor hygiene (war, prisons, starvation, natural disasters, the homeless, refugee camps). The louse takes a contaminated blood meal and the bacteria proliferate in its intestinal epithelium. After 3-5 days, the infected cells burst. The intestine and faeces contain very large numbers of the bacteria. The haemolymph of the insect turns red from the passage of the intestinal contents (blood) into the body cavity. The louse itself does not survive infection with R. prowazekii and dies after 1 to 3 weeks. It does not form a reservoir. The American flying squirrel (Glaucomys volans) is a sylvatic reservoir for R. prowazecki with occasional transmission to humans after aerosolization of its faeces containing infected fleas and lice. Squirrel fleas (Orchospea howardii) will bite humans and transmit epidemic typhus to humans if their normal host, the flying squirrel, is unavailable.

Note: the body louse is also the vector of recurrent fever (see borreliosis) and trench fever.
Typhus transmission via fleas

The reservoirs of endemic or so-called murine typhus (R. typhi) are rodents (mice, rats). The infection is transmitted to humans by rodent fleas such as the oriental rat flea, Xenopsylla cheopis. In certain circumstances, e.g. markets, grain stores, and forest fires, there is increased contact with rodents and their fleas and transmission can occur. In contrast to R. prowazekii, R. typhi does not kill the vector. A closely related organism, R. felis is transmitted by cat fleas (Ctenocephalides felis). One of the reservoirs for this bacterium is the opossum (California), but the organism has also been detected outside the USA, in Latin America, Africa, Europe, Thailand and New Zealand.
Cat flea. Ctenocephalides felis. Occasional vector of Yersinia pestis (plague) and vector of Rickettsia felis. Copyright ITM

**Rickettsia transmission, via ticks**
Rickettsial spotted fever and Rocky Mountain spotted fever are transmitted by the bite of hard ticks. *Dermacentor variabilis* (American dog tick) is notorious in the eastern USA, while in the western USA *Dermacentor andersoni* is the principal vector (Rocky Mountain wood tick) for *Rickettsia rickettsii*. Besides those main vectors, *Rhipicephalus sanguineus*, the brown dog tick, also plays an important role in transmitting the infection to humans in the USA and Mexico. *Amblyomma cajennense* and *A. aureoloatum* play a role in Latin America and Brazil. In Africa *Rhipicephalus* species are responsible for transmission of *R. conori* and *Amblyomma* species for *R. africae*. A wide variety of mammals constitute the reservoir. Queensland Spotted Fever, Japanese SF, Astrakhan SF, Israeli SF, Flinders Island SF and Siberian SF are also transmitted by hard ticks. *Rickettsia slovaca* was first identified in *Dermacentor* ticks from Slovakia and has subsequently been found in *Dermacentor marginatus* and *D. reticulatus* in France, Switzerland, Portugal, Spain, Armenia and Germany. The geographical areas where certain species occur, is not well known. E.g. in 2002, the first case of infection with *R. aeschlimannii* was detected in South Africa. Transmission of this bacterium can occur via the bite of *Hyalomma* ticks and *Rhipicephalus* ticks. This bacterium must of course have existed before but was previously not identified.

*Rickettsia heilongjiangensis* was isolated in 2002 from *Dermacentor sylvarum* ticks in the Heilongjiang Province of China, near the Russian-Chinese border. This rickettsia is closely related to *R. japonica*.

The bacteria enter the tick as part of its blood meal and multiply. The organisms are transmitted with the saliva during the next bite. Transovarial transmission in ticks can be 100%, but other factors also play an important role in determining the final infectious state of the vector. In the USA <1% of *Dermacentor* ticks in the wild are infected with *R. rickettsii*. This may be explained by an interference phenomenon in which infection of the tick with the very commonly occurring, non-pathogenic *R. peacockii*, *R. belli*, *R. montana* or *R. rhipicephali* prevents *R. rickettsii* from becoming established in tick ovaries. Naturally occurring double infections (two species of Rickettsia in 1 tick) have yet to be observed. Vertical transmission occurs when a female tick has infected ovaries, which ensures infected tick progeny. However, it is known that *R. ricketsii* takes a substantial toll on the tick, since few larvae emerge from infected eggs, and even fewer survive and mature into adults. Horizontal transmission depends upon transient rickettsaemia in a nonimmune host, on which uninfected ticks feed, creating newly infected ticks. Feeding adjacent (in time and space) to an infected tick allows for the acquisition of *R. rickettsii* without the presence of infection in the host (uninfected tick ingests saliva from the infected tick).
Typhus transmission via mites

Scrub typhus is caused by Orientia tsutsugamushi [Japanese “tsutsuga” = sick; “mushi” = insect]. The organism was classified in the past as Rickettsia tsutsugamushi. There are several antigenic variants (Gilliam, Karp, Kato, Shimokoshi, Kuroki, etc...). The organism is only transmitted by the bite of mite larvae known as “chiggers” (Leptotrombidium sp.). In nature the larvae feed on rats and other rodents while the adults feed on small invertebrate animals and insect eggs. The infection occurs focally in Asia where there is a specific ecological habitat of transitional vegetation (sides of roads, overgrown agricultural areas, disturbed rain forests, river banks, etc.). The larvae secrete an enzyme that dissolves animal tissue, after which the mite can suck up the fluid. This causes local irritation. When Orientia tsutsugamushi is introduced into the skin an inoculation chancre occurs in 50% of infections.

Infections with R. akari are not often seen in clinical practice and the condition “Rickettsialpox” is more of a curiosity. Transmission occurs via mite bites: Liponyssoides sanguineus. These mites parasitise mice.

Ticks that serve as vectors for Rickettsia from Eurasia, Australia and Africa.

<table>
<thead>
<tr>
<th>R. conorii</th>
<th>Rhipicephalus sanguineus</th>
<th>Mediterranean</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. sibirica</td>
<td>Dermacentor sp</td>
<td>Europe, former USSR, China</td>
</tr>
<tr>
<td>R. heilongjiangensis</td>
<td>Dermacentor sylvarum</td>
<td>China (Far East)</td>
</tr>
<tr>
<td>R. australis</td>
<td>Ixodes holocyclus</td>
<td>Queensland</td>
</tr>
<tr>
<td>R. japonica</td>
<td>Haemaphysalis longicornis</td>
<td>Japan</td>
</tr>
<tr>
<td>R. honei</td>
<td>Insufficient data</td>
<td>Flinders Island</td>
</tr>
<tr>
<td>R. africae</td>
<td>Amblyomma variegatum</td>
<td>Ethiopia, Southern Africa</td>
</tr>
<tr>
<td>R. mongolotimonae</td>
<td>Hyalomma sp.</td>
<td>France, Inner Mongolia, Africa</td>
</tr>
<tr>
<td>R. slovaca</td>
<td>Dermacentor marginatus</td>
<td>Europe</td>
</tr>
<tr>
<td>R. monacensis</td>
<td>suspected I. ricinus</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
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<tr>
<td>-------------------------------</td>
<td></td>
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<tr>
<td>R. helvetica</td>
<td><em>Ixodes ricinus</em></td>
<td></td>
</tr>
<tr>
<td>R. aeschlimannii</td>
<td><em>Rhipicephalus appendiculatus</em>, Africa</td>
<td></td>
</tr>
<tr>
<td><strong>Hyalomma marginatum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrakan fever agent</td>
<td><em>Rhipicephalus sanguineus</em> and <em>R. pumilio</em></td>
<td>Astrakhan region of ex-USSR</td>
</tr>
</tbody>
</table>

**Clinical aspects**

**General features rickettsial disease**

As there are several diseases that are caused by *Rickettsiae*, a general description is difficult. The **incubation period is 1 to 3 weeks**. After inoculation, *Rickettsiae* **proliferate intracellularly in the endothelium of small blood vessels**. Endothelial damage results in **focal occlusive endangiitis** in small venules and arterioles. Histologically this is identified in tissue sections in the form of typhus nodules (**Wolbach nodules**; not to be confused with typhoid nodules in the liver in typhoid fever!). In this way a **generalised, multifocal, multi-organ vasculitis occurs**. This can lead to thrombosis and vascular occlusion, possibly with oedema and local necrosis. As practically every organ in the body can be affected, the symptoms are extremely diverse. The various symptoms can be better understood if the localisation of the vasculitis lesions is borne in mind.
Skin rash during infection with Rickettsia conori. Copyright ITM
The lesions appear in:

- **Skin**: At the site of the arthropod bite there is sometimes a papulovesicular lesion with local necrosis: inoculation chancre (tache noire [black spot]). The regional lymph nodes can enlarge subsequently. A chancre occurs in fièvre boutonneuse, South American RMSF (“Sao Paulo tick fever”) and frequently in scrub typhus (but not necessarily). The chancre is almost always absent in North American RMSF and never present in epidemic and endemic typhus. The rash should be distinguished from severe measles, severe dengue and septicaemic purpura, e.g. due to meningococci. With a mild rash, a distinction must be made from typhoid fever (treatment differs).

- **Brain**: Meningo-encephalitis with confusion (“tuphos”), delirium and coma. Distinction from cerebral malaria is important. Often occurs with scrub typhus, epidemic typhus and RMSF. Hemiplegia can occur. In general there are features of aseptic meningitis, but in RMSF there can also be an increase in the number of neutrophils in the cerebrospinal fluid. Deafness may persist for months in scrub typhus.
• **Myocardium**: Myocarditis, heart failure, hypotension and shock. Hypovolaemia as a result of bleeding and increased vascular permeability contributes to low blood pressure.

• **Blood vessels**: Occlusion of arteries results in gangrene, possibly late onset (toes, fingers) and occurs predominantly in epidemic typhus and RMSF. Thrombophlebitis occurs as a result of vasculitis and stasis in severely ill patients.

• **Kidney**: kidney failure from vasculitis and interstitial nephritis, promoted by hypotension; albuminuria, oliguria.

• **Eyes**: Conjunctivitis, papilloedema (with cerebral involvement). Enlargement of the blind spot and scotomas occur frequently in scrub typhus.

• **Lungs**: Cough, tachypnoea, dyspnoea.

### Clinical aspects of epidemic typhus, scrub typhus and RMSF

These infections usually have a **very serious course**. The incubation period is 5-10 days for scrub typhus and RMSF and ± 12 days for epidemic typhus. After a few days of generally not feeling well, a **high fever** occurs. It is associated with severe general malaise, severe headache, muscular pain, conjunctivitis, cough, hypotension, meningeal irritation, vomiting, epistaxis, confusion or coma. Hepatosplenomegaly occurs occasionally but is rare. Lymphadenopathy occurs in approximately one in four patients. **Rash appears around the 3rd to the 7th day** after the onset of fever. The absence of a rash in the first few days often makes it difficult for the diagnosis to be suspected at an early stage. The skin rash in RMSF begins on the wrists, palms and soles and spreads **centripetally** to the trunk. In epidemic typhus and scrub typhus it is the reverse: beginning on the trunk (axilla), it spreads **centrifugally** over the rest of the body, sometimes sparing the face, hands and feet. The rash may develop into purpura and can rapidly become haemorrhagic. **Gangrene** of the fingers and toes can occur. Because of diffuse intravascular coagulation (fibrinogen consumption), there may be a pronounced bleeding tendency. Rocky Mountain fever sequelae include deafness, amputations and permanent learning disabilities.

*O. tsutsugamushi* has several subtypes and repeated infections with scrub typhus are possible. Untreated scrub typhus fever can persist for more than 2 weeks and is often accompanies by intense headache and diffuse myalgias. In about 50% of patients an eschar is present.

**Brill-Zinsser disease** is defined as the recrudescence of epidemic typhus years after the initial episode. In contrast to acute primary infection Brill-Zinsser disease is generally a mild illness.
Clinical aspects of endemic typhus

Endemic flea-borne typhus follows the same course as epidemic typhus, but milder. Reaching a clinical diagnosis is difficult and the disease is often missed. Rash occurs in half the cases. There is no chancre. Similar symptoms are present in infection with *R. felis*. Differential diagnosis includes typhoid fever, ehrlichiosis, dengue and other arboviroses.

Clinical aspects of tick-borne rickettsioses

Rickettsia spotted fever

This disease follows the same clinical course as mild RMSF. The rash is generalised. The inoculation chancre is characteristic here. During physical examination, a search for this chancre often leads to the correct diagnosis. Subcutaneous vasculitis can result in the formation of subcutaneous nodules (fièvre boutonneuse). *R. africae* occurs predominantly in Southern Africa. Skin rash is more confined or absent in infection with *R. africae*.

Clinical signs of infection consist of a skin lesion at the site of the tick bite and regional lymphadenopathy it is often painful. Fever and rash develop subsequently. The acute disease can be followed by fatigue and residual alopecia at the bite site.

Rickettsialpox

*Rickettsia akari* causes rickettsialpox. It is a rare infection which manifests as a self-limiting, febrile, vesicular skin rash, often confused with varicella. The differential diagnosis includes monkeypox, a viral pox disease which to date is endemic in Central Africa and is known to cause epidemics in men having sex with men.

Diagnosis

Clinical

In developing countries, the diagnosis of typhus is predominantly clinical. If scrub typhus (Southeast Asia) or boutonneuse fever (Africa, Mediterranean Sea basin) is suspected, a chancre should be sought.
Eschars are may be overlooked easily when a a careful clinical exam including inspection of genitalia and skin folds under the breast is not performed.

The rash should be distinguished from, among others, dengue, rat bite fever, secondary syphilis, meningococcal septicaemia, ehrlichiosis, varicella, herpes zoster, rubella, Epstein-Barr virus infection and severe measles. Q fever does not produce a rash.

In RMSF, the cerebrospinal fluid is usually normal, although sometimes the neutrophil count is slightly raised. Scrub typhus and murine typhus can cause an increased number of lymphocytes in the cerebrospinal fluid in meningo-encephalitis so that the infections can resemble (arbo)viral infections and leptospirosis. In the blood, the white blood cell count is normal or reduced.

Diffuse intravascular coagulation often occurs which is accompanied by thrombocytopenia.

**Serology**

The diagnosis can be confirmed at a late stage by serology. A 4-fold increase in titre between acute and convalescent sera must be detected. Serologic testing is helpful for a retrospective diagnosis of rickettsiosis but will not assist in clinical decision making. IgM and IgG antibodies typically appear 7 to 10 days after the onset of the illness, the optimal time to obtain a convalescent antibody titre is at 14 to 21 days after the onset of symptoms. Treatment must be started early without waiting for laboratory confirmation.

**Culture**

Isolation of the organism by blood culture is usually not performed. Culture of rickettsia is difficult, laborious and dangerous (tissue culture or isolation on embryonated eggs). *Rickettsia* is an obligate intracellular parasite and in only a few reference labs in the world it is cultured on cell culture monolayers. Growth is confirmed using specialized stains (e.g. Gimenez) Guinea pigs can be inoculated with blood from a patient. After 4-5 days, the animals develop fever and male guinea pigs develop a swollen scrotum (Neil-Mooser reaction). There is a significant risk of laboratory infection.

PCR technology has become very important in identifying rickettsial species and strains: this is usually done on blood or skin biopsies of eschars.


## Treatment

Untreated, the mortality of RMSF is 20 to 40% and of epidemic typhus ± 20%. General status (malnutrition, etc) plays a role here. Scrub typhus has a mortality rate of 6%, endemic typhus follows a milder course (mortality 2%) and fièvre boutonneuse has a low mortality (< 1%). It is not necessary to wait for confirmation of the diagnosis for treatment. If the course is fulminant, antibiotics are relatively ineffective.

### Antibiotics

**Tetracyclines** are active against the organisms and are the first line treatment. The organisms are not 100% eliminated from the body. Recovery is determined by the patient’s immunological resistance. Doxycycline is very useful in epidemics of louse-borne typhus and for scrub typhus. RMSF and endemic typhus should be treated for at least 1 week. In epidemic typhus an improvement may be expected within 24 to 72 hours.

**Chloramphenicol** is second choice, e.g. in pregnancy, notwithstanding the risk of “grey-baby” syndrome. **Ciprofloxacin** has some activity against Rickettsiae, but is inferior to doxycycline. Azithromycin has been used for mild forms in pregnancy. Erythromycin is not a good choice. Often the initial differential diagnosis includes bacterial meningitis caused by *Haemophilus influenza* or *Neisseria meningitidis*. Chloramphenicol is also active against these organisms. **Penicillins, ampicillin and streptomycin are inactive against Rickettsiae**. Traditionally it is assumed that scrub typhus is highly susceptible to tetracyclines (this is sometimes used as a diagnostic test). In Thailand in 1996, scrub typhus infections were observed which clearly exhibited reduced susceptibility to doxycycline (both clinically and in vitro). Azithromycin or rifampicin 900 mg daily for 1 week is used as treatment in these cases.

### Vector control

All patients and their clothing should be free from insects, ticks and mites. **Delousing** is of major importance in epidemics. The patient should be washed (removal of louse faeces on the skin and in the hair). Clothes and sheets should be decontaminated.

### Prevention

Epidemic typhus: Delousing (e.g. 1% permethrin or 1% malathion puffs in/on clothing, heat sterilisation of clothing), treat cases, improve general hygiene.
Endemic typhus: Rodent control

Scrub typhus: Preventive antibiotics, rodent control. DEET or permethrin on clothing and skin. In endemic areas vegetation must be cleared.

RMSF and rickettsia spotted fever: Protective clothing, tick repellents in infested areas. Manual removal of ticks

### Weigl vaccine

The so-called Weigl vaccine was produced from 1920 to 1930. Lice were inoculated intrarectally with viable *R. prowazekii*. The lice fed on Dr Weigl and on the bodies of his colleagues so that the rickettsia was able to proliferate. Some of his colleagues died from typhus. Some 100 lice were necessary for one dose of vaccine. Subsequently it was decided to culture a louse strain ("Orlando") that sucked blood from rabbits. This strain is still the reference strain for study of these insects.

Q-Fever

### General

In some textbooks, Q fever is included among the rickettsioses for historical reasons, but clinically the condition differs fundamentally from “typhus” presentations. In 1937, Derrick described a new and unusual febrile illness affecting abattoir workers in Brisbane, Australia. When the blood of these febrile patients was injected into guinea pigs the animals developed mild fever and splenomegaly. Burnet identified small, filterable rickettsial-like micro-organisms in the spleens of these infected animals. Cox cultured the bacteria in yolk sacs of embryonated hen’s eggs (the bacteria cannot be cultured on cell-free media). Davis and Cox isolated the organism from ticks collected near Nile Mile Creek in Montana, USA. The disease is now called Q fever, where the Q refers to query because of the initial mysterious nature of the disease. Cox and Burnet have been honoured for their discoveries in the designation of the causal agent *Coxiella burnetii*.

*Coxiella burnetii* is a small (0.3-1.0 µm) pleomorphic strict intracellular Gram-negative
**bacterium** that originally was classified among the *Rickettsiaceae*. More recent phylogenetic studies show that taxonomically the organism is only distantly related to the *Rickettsiaceae*. Gene-sequence analysis (16S rDNA) now classifies it in the order of the *Legionella*, family *Coxiellaceae*.

*C. burnetii* proliferates **intracellularly in an acidic vacuole** (phagolysosome, pH 4.8). Infection with this bacterium inhibits the normal final phagosome maturation step, and therefore the bacterium will survive. Interferon-gamma reverses this and allows intracellular killing of the bacterium. Interferon-gamma also induces the killing of *C. burnetii* through apoptosis of infected macrophages. The organism can survive for a long time as a spore (small-cell) in very unfavourable conditions in the environment. The small-cell and large-cell variants can be distinguished by electron microscopy. The large-cell variants multiply in host cells. These variants should not be confused with antigenic states phase I and II (see further).

**Epidemiology**

**Reservoir**

The reservoir is found in animals. Q fever is a **worldwide zoonosis**, although no endemic cases have occurred in New Zealand. Arthropods, fish, birds, rodents, marsupials, horses, dogs, cats, cattle, goats and other animals can be infected. The most important sources of infection for humans are cattle, sheep and goats. In these animals, the uterus and mammary glands are primary sites in the chronic phase of infection. Infected mammals shed bacteria in urine, faeces, milk and birth products. High concentrations of *C. burnetii* (up to 10^9 bacteria per gram of tissue) can be found in the **placenta of infected mammals**. Bacterial spores can remain viable in **dust and dried faeces** for a very long time (years).

**Transmission**

Transmission **between animals often occurs through ticks**. There is often reactivation of an infection in pregnant animals. During parturition, an infectious aerosol can be formed. Inhalation of contaminated aerosols from parturient fluids of infected livestock is important. Animal-to-human transmission of the infection then occurs **aerogenically**. There is apparently no human-to-human transmission. Very rarely transmission occurs from drinking contaminated milk. Inhalation of stirred up contaminated dust (e.g. sleeping in stables previously occupied by sheep, manure) is another risk factor. Persons most at risk for infection are farmers, people living downwind from farms and contaminated manure, straw or dust, laboratory personnel working with *C. burnetii* and abattoir workers.
The largest outbreak ever recorded started in Herpen, in the south of the Netherlands in 2007. It soon spread to two Dutch provinces Noord Brabant and Gelderland. Before 2007, about 15 cases per year were diagnosed in the country. This increased to 2357 human cases in 2009, luckily with “only” 6 deaths in this year. The epidemic continued in 2010. A considerable number of cases were urban. An official ban to spread manure from goat and sheep farms did not seem to achieve significant results. Other hygienic measures, particularly pregnant women avoiding contact with small ruminants have been applied. Limited vaccination of milking sheep and goats was undertaken in 2008. A massive vaccination program was undertaken in 2009 (see further, under prevention).

Q fever was studied by the military for its potential as a biological weapon.

**Clinical aspects**

Primary infection with *C. burnetii* is commonly asymptomatic. HIV patients appear to have a higher risk for symptomatic disease. The incubation period is rather long (14-26 days with an average of 15 days). Q fever does not cause direct vasculitis and the infection manifests itself differently from typhus. However, circulating immune complexes may occur which can lead to glomerulonephritis and leukocytoclastic vasculitis. There is no such thing as “classic Q fever”. Most symptomatic patients have a self-limiting, febrile syndrome, possibly with headache, nausea and losing weight ± atypical pneumonia; similar to *Mycoplasma pneumoniae, Chlamydiae pneumoniae*, legionellosis or viral pneumonia. With pulmonary involvement, there is often no cough (cough occurs in only 25%), but in general the chest X-ray will be abnormal. Hepatitis and endocarditis also occur, as well as - albeit rarely- thyroiditis, orchitis, pancreatitis, myocarditis, pericarditis, SIADH, haemophagocytosis or erythema nodosum. Various neurological problems can occur, including optic neuritis and aseptic lymphocytic meningitis. In Q fever cerebrospinal fluid is often normal even though *C. burnetii* can be isolated from patient’s cerebrospinal fluid. Encephalitis and/or cerebellitis can occur (often with ataxia). Severe headache and chronic tiredness are also frequently present. There is rarely rash and there is no chancre. Sometimes slight leukocytosis is present, but in most cases (75-90%) the white blood cell count is normal. Thrombocytopenia is present in approximately 1/3 of patients. Liver enzymes and creatine kinase levels can be elevated.

Cases of Q fever have been reported in pregnancy. Intrauterine transmission has been documented. The placenta can develop necrotic foci (vasculitis) and fetal infection is known. There is an increased risk of oligamnios, fetal miscarriage, abortion, prematurity, low birth weight and neonatal death. There is also a risk to the obstetrician delivering the baby. Long-term treatment with cotrimoxazole protects against maternal chronic Q fever, although it is only bacteriostatic and carries the risk of neonatal hyperbilirubinemia if used just before delivery.
Chronic Q fever develops in a minority (1-5%) and is defined as infection lasting for 6 months or more. The organs most commonly affected are heart, arteries (vascular aneurysm), bones (beware prothesis, osteomyelitis) and liver. In rare cases mixed cryoglobulinemia can occur. Chronic disease may develop insidiously months or years after the acute disease. In chronic Q fever with cardiac valve involvement, vegetations are only rarely found on echocardiography. Q fever endocarditis carries a high mortality and tends to occur in patients with pre-existing valvulopathy. Chronic Q fever is most likely to develop in those who are pregnant, immunocompromised (eg, patients receiving prolonged or high-dose corticosteroid therapy or tumour necrosis factor-alpha inhibitors), have underlying valvular or vascular disease or a prosthetic joint. In such patients, C. burnetii multiplies in macrophages and produces a prolonged bacteraemia; the resulting high levels of antibodies and immune complexes directed at the organism contribute to many of the symptoms.

**Diagnosis**

The diagnosis is extremely difficult and based on specific serology. The best approach is to look for seroconversion. IgM can remain positive for a very long time, even longer than one year in this infection (low titres). The serological response in acute infections is mainly IgM against phase II antigens, followed by IgG antibody to phase II antigen. In chronic infection there is a serological response (IgG and to a lesser extent IgA) to phase I and II antigens. Phase I antigens are less immunogenic than phase II antigens. In patients convalescing from acute disease, phase I antibodies decrease rapidly. In patients with chronic disease, antiphase I titres remain raised as a consequence of constant antigenic stimulation. Immunofluorescence titres to phase I antigen of 1/800 or more are very suggestive of endocarditis, but the cut-off titres used in different labs are variable. As such a positive serology is a major criterion in the “modified Duke criteria” for endocarditis. Because of cross-reactivity between Coxiella and Bartonella antibodies, a positive Bartonella-serology in a patient in whom Q-fever endocarditis is suspected, paradoxically favours the diagnosis of Q-fever. Remember: cats are sources of both C. burnetii and B. henselae. PCR can be performed on excised heart valve tissue or serum in the initial stage of the infection when serology reveals no or low level antibodies.

Diagnosis chronic Q fever:

1. Phase I IgG larger than or equal to 1/4096, or
2. Phase I IgG larger than phase II IgG, or
3. PCR Coxiella burnetii positive after one month of illness
Antigenic variation

*Coxiella burnetii* displays antigenic phase variation, similarly to the smooth and rough colonies of certain bacteria when they are cultured in Petri dishes. In animal or human infection, *C. burnetii* exhibits phase I and is very infectious, but after repeated passage in cells or embryonated eggs, it converts to the non-infectious phase II. This transition is associated with a chromosomal deletion. Phase I antigen is a polysaccharide component of lipopolysaccharide. When some carbohydrates are lost, phase II antigen appears. In acute Q fever antibodies against phase II predominate, but in chronic fever the highest titres are found against phase I antigens.

Suggestive but transient “doughnut”-shaped granulomas (fibrinoid ring formation) are sometimes detected by liver biopsy. In practice, most cases of Q fever are missed unless serology (IgG and IgM) is available. Culture is possible in embryonated hen’s eggs and in various cell lines (human embryo fibroblast cells, green monkey kidney cells and others). However, in view of the infectious and dangerous nature of the organism, in vitro isolation is rarely performed. People who work (e.g. research) with *Coxiella burnetii* have an increased risk of becoming infected.

Treatment

The aim of treatment is different in acute and chronic Q fever.

In acute infection, bacteriostatic treatment will usually suffice for a clinical cure. Doxycycline is a good choice here (200 mg/day x 2-3 weeks). Clarithromycin or azithromycin are alternatives.

In chronic Q fever, a bacteriostatic treatment will probably control the disease but not cure it. Bactericidal therapy is preferable. Since the organism lives in a very acidic environment (pH of the phagolysosome = 4.8), an attempt may be made to alkalinise the vacuole, for example by the simultaneous administration of hydroxychloroquine. This will raise the pH from 4.8 to 5.7. In this way it is possible to render doxycycline bactericidal. The preferred treatment for chronic Q fever is hydroxychloroquine combined with doxycycline for at least 18 months (longer if antibody titre IgG remains > 1/800). QTc-time prolongations should be monitored.

In case of Q fever endocarditis, cardiac surgery will often be required. In pregnancy, treatment with cotrimoxazole will prevent fetal death and miscarriage, but this treatment will not prevent the development of chronic infection in the mother. Once the child is delivered, treatment with doxycycline plus hydroxychloroquine for one year will enable normal subsequent pregnancies.
Prevention

When an outbreak is identified, transport of manure in the area will be prohibited.

A formalin-inactivated whole cell vaccine from the Henzerling strain (Q vax) has been used in Australia. In November 2005, CSL Ltd in Australia (Commonwealth Serum Laboratories, the only producer in the world) announced to stop production of the vaccine for economic reasons, but the Australian government subsequently prevented this. In the Soviet Union, an avirulant variant of the Grita strain has been studied as a vaccine. However, the general public does not need to be vaccinated. Vaccination of people at risk (e.g. lab personnel) is useful. Prevaccination testing is advised, and includes history, serology and a skin test with dilute vaccine. In order to stem the large Dutch outbreak of 2007-2011, the Dutch government provided 400,000 vaccine doses in 2009 (Coxevac, a killed vaccine based on the Nile Miles strain).

Vaccination for humans are reserved for high risk professions (e.g. slaughterhouse workers) and patients with

1. previous endocarditis (non-Coxiella)
2. heart valve prothesis
3. congenital heart disease
4. aortic or mitral valve problem
5. aortic aneurysm
6. aorta prothesis

Contraindications for vaccination with Q-vax include pregnancy and previous Q-fever.

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Ehrlichia and Anaplasma

_Ehrlichia_ and _Anaplasma_ are bacteria related to _Rickettsiae_. They are obligate intracellular bacteria that grow within membrane-bound vacuoles in human and animal leukocytes. The obligate intracellular bacteria proliferate in white blood cells: monocytes (_E. chaffeensis_, HME: human monocytic ehrlichiosis) or granulocytes (_Ehrlichia_ sp. related to _E. equi_ (horses) and _E. phagocytophila_ (cattle)).

**Historical note**

The generic name refers to Paul Erhlich (1854-1915), the famous German bacteriologist (Nobel Chemistry Prize 1908), the discoverer of salvarsan, an arsenical preparation active against syphilis. In 1954 the first human ehrlichiosis was described in Japan, caused by _E. sennetsu_. Since this initial report, several tick-borne infections have now been recognized. Human monocytic ehrlichiosis (HME) was first described in 1986 and is caused by _Ehrlichia chaffeensis_. The name refers to the American army base Fort Chaffee in Arkansas.

Human granulocytotropic anaplasmosis (HGA) was described in 1993 and is caused by _Anaplasma phagocytophilia_. _Ehrlichia ewingii_ was described in 1999 as an agent of human ehrlichiosis. _E. ewingii_ provokes “human granulocytic ehrlichiosis.”

**Transmission and infection**

The organisms are transmitted by ixodid ticks. _Amblyomma americanum_ (Lone star tick) is the main vector for _E. chaffeensis_. In the USA white-tailed deer and coyotes form the most important reservoir. It has been shown that ticks on migrating birds can be infected with _Erhlichia_ sp. and can thus be transported over long distances. _Anaplasma phagocytophilia_ in the broad sense is found in rodents such as the dusky-footed wood rats and mice. The reservoir of _E. ewingii_ is still unknown.

Transmission occurs predominantly by the bite of infected ticks, but mother-to-child transmission and transmission by blood transfusion or slaughtering of infected animals is reported.

Common symptoms include fever with or without chills, headache, myalgia, arthralgia, weakness, nausea, leukopenia and thrombocytopenia. Rash is uncommon. Liver test abnormalities can be found in about 50% of cases. In rare cases human monocytic ehrlichiosis can be associated with neurological lesions or meningitis. Post-infection asthenia can continue for months. In HIV patients
infection can be overwhelming.

**Diagnosis**

White blood cell and platelet abnormalities are almost always present, so normal values virtually rule out this infection. Anemia is commonly present, so pancytopenia can be suggestive of anaplasmosis or ehrlichiosis.

Probably many infections are missed since laboratory testing is not widely available. The diagnosis of human granulocytic anaplasmosis is made by microscopic examination of a peripheral blood smear or serologic testing. A 4-fold rise in antibody titer between the acute an convalescent phases of infection confirms the diagnosis. Microscopy is labor intensive and the sensitivity of microscopy ranges from 20 to 80% depending on the degree of expertise: bacteria are observed in the cytoplasm of leukocytes as 0.5 to 1.5 µm large inclusions which are combined in groups (morulae). Today, PCR has gained diagnostic importance in high resource settings. Culturing of this intracellular bacteria is complex and it’s the most accurate method, and it is reserved for research purposes.
Figure 2. Bone Marrow–Biopsy Specimen, Bone Marrow Aspirate, and Peripheral-Blood Specimen.

Hematoxylin and eosin staining of a bone marrow core-biopsy specimen (Panel A) and Wright–Giemsa staining of a bone marrow aspirate smear (Panel B) show maturing trilineage hematopoiesis. On the bone marrow aspirate smear (Panel B) and on Wright’s staining of a peripheral-blood smear (Panels C and D), most neutrophils show nonspecific toxic granulation; rare ones have intracytoplasmic inclusions (arrows), which are suggestive of human granulocytic anaplasmosis.

**Differential diagnosis:**

The differential diagnosis includes rickettsiosis, typhoid fever and several arboviral infections, such as dengue.

The diagnosis of HGA can be overlooked if there is simultaneous infection with *B. burgdorferi*. In such cases, the typical rash of early Lyme disease (erythema migrans) may mislead the clinician into ignoring possible coinfection with ehrlichia or anaplasma. Findings that may suggest coinfection
include leukopenia, thrombocytopenia, and high fever (all relatively uncommon in Lyme disease) and abnormal liver enzyme tests accompanying the erythema migrans.

**Treatment**

Treatment is based on administration of tetracyclines, e.g. doxycycline 100 mg twice daily for 7 days.

**Carrion’s disease**

**History**

South American-bartonellosis, (Carrión’s disease, Oroya fever, verruga peruana, verruga peruviana) results from infection with the bacterium *Bartonella bacilliformis* and is transmitted by *sandflies*. The infection manifests itself in two very different clinical forms with the causal connection being recognised by the young Peruvian doctor Daniel Alcides Carrión.

Pre-Columbian mummies with histologically confirmed verruga lesions have been discovered in Peru and bartonellosis occurred in Francisco Pizarro’s army (1471-1541). During the Inca era, the disease was called “Sirki,” which means “warts in blood.” In Peru between 1869 and 1873 more than 7000 workers building the Lima-La Oroya railway died from the disease at Cocachacra, 65 kilometers from Lima, 1600 meters above sea level. The name “Oroya fever” refers to this, although in the mining town of La Oroya (altitude 3800 m), strangely enough there was no transmission of Oroya fever. In 1936 a large epidemic was seen in the Guaitara valley on the border between Colombia and Ecuador. An epidemic occurred in 1980 in Ecuador and another in 1987 in Peru with a death rate of 88% in the untreated patients. Now and then there have been isolated cases or small outbreaks. In 1997 there was an outbreak in the area of Cuzco, Peru. In an outbreak in Zumba, Ecuador (1995-96), large numbers of dead rodents were found around the places where the cases had occurred. This finding led to the hypothesis that bartonellosis could have an animal reservoir.

**Daniel Alcides Carrión**

Daniel Alcides Carrión (1858-1885) was a medical student in Lima, Peru. He was required to prepare an original thesis and choose to study the epidemiology and clinical manifestations of verruga peruana. His home was in Cerro de Pasco, a mining town high in the Andes where he had
seen many cases. These left a deep impression on the young man. He told a classmate that he hoped “to make an important contribution to aching humanity”. He became concerned with the difficulty in diagnosing verruga peruviana before the typical eruption started. The appearance of the skin lesions was preceded by fever and anaemia, but there was a lot of confusion between the prodromal phase of verruga and other febrile disorders such as malaria. Carrión wanted to determine the incubation period and early symptoms of verruga, so he decided to inoculate himself with some fluid from a chronic skin lesion of a verruga patient. Many friends and professors tried to dissuade him. On the morning of August 27, 1885, Carrión was in the Nuestra Senora de las Mercedes ward of the Dos de Mayo Hospital in Lima. A 14-year-old boy named Carmen Paredes was admitted with verruga on his right eyebrow. Assisted by Dr Chavez, a young ward physician, Carrión used a lancet to inoculate his own arm with blood taken from that verruga. He kept a diary afterwards. The first symptoms started after 21 days, with discomfort and pain in his left ankle. Two days later he developed fever, chills, abdominal pain and generalised pain in bones and joints. He had anorexia and noted severe thirst. His urine became dark red and scanty. He developed jaundice. A week later, he became too ill to continue his diary. His classmates took over this task and were surprised at how quickly anaemia developed. A systolic heart murmur developed and grew in intensity. A few days later, muscle fasciculations appeared in his arm muscles. He said to his friends: “Up to today, I thought I was only in the invasive stage of the verruga as a consequence of my inoculation, that is, in the period of anaemia that precedes the eruption. But now I am deeply convinced that I am suffering from the fever that killed our friend, Orihuela. Therefore, this is the evident proof that Oroya fever and the verruga have the same origin, as Dr Alarco once said.” This insight was the essence of Carrión’s experiment. He had not set out to prove the single cause of verruga peruviana and Oroya fever. He only intended to study the incubation period and prodrome of verruga. When a completely different disease developed, he was lucid enough to understand the full meaning of his experiment. On October 3, he became delirious and two days later he fell into a coma and died at midday. He became a hero of Peruvian medicine and is remembered to this day. The day of his death, October 5, is celebrated yearly as the “Dia de la Medicina Peruana”. The Peruvian National University in Cerro de Pasco carries his name.
Ponte verrugas in the Andes, a railway bridge on the trail Lima – La Oroya (Peru). The name refers to a bartonellosis epidemic in 1869-1873. Copyright ITM

Picture of Dr Daniel Alcides Carrion, on the road Lima – La Oroya. In this area of the Western Andes in Peru, there was a bartonellosis epidemic in 1869-1873. The disease is also known as Oroya fever or Carrion’s disease. Photo Dr Van den Enden. Copyright ITM

### Aetiology

Barton described the pathogen in 1909, but he thought that it was a protozoon. The Japanese bacteriologist Hideyo Noguchi demonstrated the bacterial nature of the pathogen. *Bartonella bacilliformis* is a **small Gram-negative coccobacillus** (0.6-1 µm), which takes Giemsa and Warthin-Starry stain. The pathogen has one or more polar flagella. It replicates within the vascular endothelium and erythrocytes. The bacterium is **related to rickettsiae**. The bacillus grows quickly (extracellularly) on non-living culture media with blood or on chicken embryos at 25-28°C. Numerous
related organisms are animal pathogens.

**Distribution**

The disease caused by *Bartonella bacilliformis* only occurs in certain narrow high valleys of the western-most slopes of the Andes at altitudes between 500 and 3200 meters in Peru, Ecuador and Colombia, between 2° N and 13° S. Whether endemic cases occurred in Chili, Bolivia, Guatemala and Honduras is very doubtful. Sporadic cases of so-called “bartonelloses” have been reported in Africa, (Niger, Sudan), in Asia (Pakistan) and in the USA, but it is still not clear whether there is a connection with Carrión’s disease. Our knowledge about *Bartonella* and related bacteria has largely increased in recent years but is still very incomplete.

**Transmission**

A sandfly, *Lutzomyia verrucarum*, and perhaps a few related species, is responsible for transmission. Transmission only occurs at night and is seasonal, particularly during the rains. It was formerly assumed that the reservoir was purely human, but this was recently cast into doubt (there may be a rodent reservoir). In some of the inhabitants in the endemic valleys bacteria can be found in the blood, but these carriers are usually without any symptoms. These latent infections which are likely to have been contracted in childhood probably give stable immunity. It is only if non-immune populations enter the endemic area that epidemics occur, sometimes on a large scale, such as in wars or when large public works are being carried out. Tourists may be at risk for the disease.

**Clinical aspects**

The clinical range is wide, going from asymptomatic infections via serious febrile forms with acute haemolytic anaemia, to the angiomatous skin lesions which can be present from the onset or can be preceded by the febrile stage. The mortality of untreated cases varies between epidemics and ranges from 10-40% after 2-3 weeks. The disease is less severe in children and the mortality is far lower. If the course of the disease is favourable, the fever can last for 3 to 4 months. In 40-50% of cases of Oroya fever, concurrent salmonellosis (generally Salmonella typhimurium) complicates the illness and makes the prognosis less favourable. The superinfection causes fever with gastrointestinal symptoms and a deterioration of the patient’s general condition.

**Acute stage or Oroya fever**

1. Incubation takes approximately 3 to 8 weeks (range 10-210 days). It begins insidiously with:
2. Irregular **intermittent febrile** attacks with shivering

3. Rapidly worsening **anaemia** with tachycardia, pallor and (sub)icterus

4. Severe **headache** with bone and joint pain. This may persist after the fever has ended

5. Enlargement of the **liver and spleen**, slightly painful on palpation

6. Generalised painful **swollen lymph nodes**

7. Myocarditis, pulmonary oedema and anasarca (generalised oedema)

8. **Haemorrhagic diathesis** as a result of the endothelial lesions: petechiae and tendency to thrombosis. Necrotic foci are found in the liver, spleen and bone marrow.

9. **Neutrophilia**

10. Spontaneous abortion, foetal death or transplacental transmission can occur.

11. **Neurobartonellosis** due to involvement of the CNS takes the form of meningo-encephalitis with or without convulsions and with high mortality. Myelitis also occurs with spastic or flaccid paraplegia with sequelae which can be permanent. There is pleiocytosis of the CSF. More focal and transient lesions of the spinal cord or of the cranial nerves are seen at the verruga stage.

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**Verruga peruviana**

This is the chronic eruptive stage of infection with *Bartonella bacilliformis*. The painless wart-like skin eruption results from the abnormal growth of blood vessels with the appearance of haemangiomias and the formation of angioblastic nodules. At this stage *Bartonella* can still be found in the endothelial cells, but they are only very rarely found in the erythrocytes.
Verruga peruviana in chronic bartonellosis (infection with Bartonella bacilliformis). Do not confuse this lesion with a granuloma pyogenicum. Copyright Alexander von Humboldt Institute, Peru
Bacillary angiomatosis. Ulcer due to infection with Bartonella henselae. Copyright Alexander von Humboldt Institute, Peru

The skin eruption usually appears **6 to 14 weeks after the acute stage**. Both pathological conditions can be present at the same time. The skin eruption may initially be accompanied by a mild fever and arthralgia. The eruption is polymorphic. Some lesions disappear quickly, others persist or grow for some time only to shrivel and disappear, generally without leaving scars. There are three forms:

**Miliary** form: the lesions are small (< 0.5 cm), very numerous and mainly found on the face, on the extensor surface of the limbs and on the trunk. They are initially macular and grow to small vascular, sometimes pedunculated and protruding nodules. Lesions are also present on the digestive and genito-urinary mucosa. Dysphagia, haematemesis, melaena, haematuria and metrorrhagia can occur.
**Nodular** form: the nodules are larger, less numerous, deeper, chronic and mainly found around the elbows and knees. The mucous membranes are spared. The lesions appear in cycles for 2 to 3 months.

**Mular** form: there are isolated pseudotumoural haemorrhagic nodules which macroscopically resemble granuloma pyogenicum.

**Immunity**

Immunity is **gradually acquired during the acute stage**, so that the disease becomes **limited to the wart-like lesions** of the skin and mucous membranes which subsequently heal completely and permanently. In experimentally infected monkeys **the disease can be reversed** from the verruga stage to the febrile haemolytic stage by splenectomy. The same probably occurs in humans. The prognosis of verruga is good. They evolve in spurts and the lesions generally heal spontaneously in less than 6 months.

**Diagnosis**

The diagnosis is based on the endemicity, the clinical characteristics, full blood count, the presence of *Bartonella* in blood smears (Giemsa stain), blood cultures or tissue cultures from skin lesions or even the histological examination of the latter. More than 70 percent of patients with acute Oroya fever have a positive blood culture for *B. bacilliformis*, although there may be a delay of more than 14 days for the organism to grow in culture. *B. bacilliformis* is fastidious and requires Columbia agar, an enriched blood medium, for growth, which occurs most readily at 25 to 28°C.
Bartonella bacilliformis in red blood cells

In the differential diagnosis, consideration is given to malaria, dengue, viral hepatitis, babesiosis, bacillary angiomatosis in AIDS patients, typhus, typhoid fever, Yaws, Kaposi’s sarcoma, haemangiomas, pyogenic granuloma and various skin tumours. In mild forms, the number of Bartonella in the blood smear can fall below the detection limit. The degree of haemolysis is then very limited, and the infection is extremely difficult to diagnose if no serology is available. PCR, Immunofluorescence, ELISA and Western Blot among others are used for diagnosis.

**Treatment**

Until recently **chloramphenicol** was the drug of choice but **ciprofloxacin** has now been shown to give better results. Both are also effective in *Salmonella* infections (in absence of resistance). Chloramphenicol is administered at doses of 4 g/day for 5 days. Ciprofloxacin is given as 500 mg BD. The fever disappears in less than 48 hours. The mortality rate of Oroya fever can be largely reduced with antibiotic therapy. Late development of the verruga stage is possible despite correct treatment.
Ciprofloxacin or rifampicin for 2 to 3 weeks can be used in the verruga stage.

**Disease control**

Spraying with insecticides, especially those which retain their activity for long periods interrupts transmission. However, control measures are not essential in endemic areas because of the immunity of the adult population. Individual protection consists of avoiding spending the night in exposed biotopes and the use of insect repellents or mosquito nets treated with permethrin/deltamethrin. Although theoretically possible vaccination is not used.

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**Bartonella quintana**

**General**

*Bartonella quintana* is a very small Gram-negative rod-shaped bacterium, responsible for a whole range of infections. Infections with this bacterium occur where *people are living in wretched circumstances and are infested with lice*. Trench fever was the first clinical manifestation of infection with *Bartonella quintana* to be recognised. The name refers to its association with the German and Allied troops in the First World War. It is estimated that more than one million people were infected during the war. British troops took the disease to Mesopotamia at the time of Lawrence of Arabia. After the war the incidence fell very sharply. The disease broke out again during the Second World War with large-scale epidemics. As the taxonomic understanding improved over the years, the pathogen underwent several name changes: *Rickettsia quintana*, *Rickettsia weigli*, *Rochalimaea quintana* and finally *Bartonella quintana*.

The 1.6 Mb genome of *Bartonella quintana* has been sequenced. It is closely related (maybe a degenerative form) of *B. henselae*, which itself can be considered a shortened version of the *Brucella melitensis* genome.

**Transmission**

The natural reservoir is still uncertain. The body louse *Pediculus humanus corporis* is the vector. These insects bite an average of 5 times per day. The bacteria multiply in the lice. *Bartonella quintana* survives up to a year in louse faeces. Since B. quintana propagates in the intestinal lumen of the body louse not in the intestinal epithelial cells, infection probably results from contact with contaminated
louse faeces. **Wounds caused by scratching** facilitates the entry of the **bacteria contained in louse faeces.** *Bartonella quintana* has also been detected in *Pulex irritans* fleas, cat fleas, cat dental pulp, monkey fleas, and has been isolated from *Pediculus humanis capitis*, the human head louse. The significance of this latter finding is still unclear. More study of possible reservoir hosts is clearly needed. It is possible that an important animal reservoir might be identified.

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**Clinical aspects**

The clinical spectrum of trench fever was described in 1919 via experimental infections in volunteer soldiers. In 1949, an accidental epidemic among 104 laboratory workers resulted in 90 symptomatic cases, which was described in detail. The incubation period varies from **15 to 25 days** (sometimes extremes of 3-38 days are mentioned). Infection can lead to several distinct clinical forms:
The patient may have no or very few symptoms. They may be subfebrile and may have symptoms of pharyngitis. People can be asymptomatic carriers and act as a reservoir. In 1995 *B. quintana* was found in 14% of the homeless in Marseilles, who presented without symptoms or with general vague aspecific symptoms.

**Chronic endocarditis** can occur. The main characteristics are fever, haematuria, splenomegaly and heart murmurs. The symptoms can be divided into (a) symptoms of infection such as fever, weight loss, malaise, nocturnal sweating, clubbing, enlargement of the spleen, anaemia and mycotic aneurysms, (b) heart murmurs and heart failure, (c) embolic phenomena such as CVA or a peripheral arterial embolism, (d) vasculitis such as microscopic haematuria with or without renal failure, splinter haemorrhages under the nails, Osler's nodules (painful lesions on the fingers), Roth's spots on the retina.

**Classical trench fever.** The patient develops fever which persists for 5 days. This is accompanied by severe headache and muscle pain, particularly in the legs ("shin pain"). Retro-ocular pain, red conjunctivae, enlargement of the spleen and leukocytosis can occur. After a fever-free interval, the fever can return. These cycles can recur 3-5, even up to 8 times. The term “quintan fever” derives from the recurring five day attacks. Mortality is very low. The pathogen may be present in the human body long after the symptoms have disappeared.

**Continuous fever** can develop for several weeks (typhoidal form), accompanied by splenomegaly and a fleeting rash (roseola).

The pathogen can be isolated from cutaneous angio proliferative skin lesions in patients with bacillary angiomatosis (*Bartonella henselae* can also be cultured from similar lesions). Many of these patients are immune-deficient (HIV). The pathogen is phagocytosed by endothelial cells and survives in a vacuole. Angiogenic factors are secreted either by the pathogen itself or by the host’s response to infection. This leads to proliferation of endothelial cells, with typical neovascularisation. **Bacillary angiomatosis** is characterised by the emergence of a few to hundreds of skin lesions, from a few mm to several cm in diameter. They are reddish purple may be ulcerated and then resemble a pyogenic granuloma or Kaposi’s sarcoma. The lesions bleed heavily when injured. They can also affect the lymph nodes, bone, bone marrow, liver and spleen. The growth of new blood vessel cells resembles the late stages of the skin lesions of verruga peruviana triggered by *Bartonella bacilliformis*. The pathogen can be detected by Warthin-Starry staining. **Peliosis hepatitis** is a condition in which numerous, blood-filled cystic spaces appear in the liver.
Diagnosis

The pathogen can be cultured axenically (in absence of all other bacterial contamination) but this takes a long time (up to 45 days). It is best to use a combination of cultures on solid medium, liquid medium and cell cultures. Since Bartonella is a facultative intracellular bacterium, the lysis-centrifugation system (Isolator) is recommended for the cultivation of Bartonella sp. from blood. Inoculation of material from the Isolator tube and from tissue onto freshly made chocolate agar plates facilitates growth of the organism. Incubation in a humid atmosphere with 5% to 10% CO\textsubscript{2} for several weeks is required for isolation. Serologically, antibodies display a great deal of cross-reactivity. Cross-absorption is indicated before performing any serological tests. Indirect immunofluorescence is the reference method. IgG of > 1/50 indicates *Bartonella* infection. Endocarditis patients usually have titres of > 1/800. It is sometimes possible to reveal the bacteria in biopsy material using a Warthin-Starry stain (a complex silver stain) or immunohistochemistry. At present, PCR has a central role.

Treatment

Not much is known about this pathogen. In vitro it is susceptible to beta-lactam antibiotics and it can also be killed in vitro by gentamicin, doxycycline, rifampicin, erythromycin and the new macrolides. To treat classical trench fever, once-daily administration of azithromycin or doxycycline is recommended. In treating endocarditis, it is preferable to use doxycycline with gentamicin or rifampicin as well as considering surgery. Bacillary angiomatosis takes 4-12 weeks to treat.

Cat-scratch disease

This disease manifests itself mainly as a rather slow-healing ulcer with chronic lymphadenitis (98%) or rarely as a systemic condition (2%). An ulceroglandular syndrome which must be distinguished from tularemia, mycotic and mycobacterial infections. Sometimes there is Parinaud’s oculoglandular syndrome (which can resemble sarcoidosis) or one of the rarer forms, such as retinitis with papilloedema. The condition is caused by *Bartonella henselae* and very rarely by *Afipia felis*. The latter pathogen derives its name from the “Armed Forces Institute of Pathology in the USA, where the bacterium was first identified in 1988. Infection is contracted by cat scratches or bites and possibly also by infected cat fleas. *Bartonella henselae* has also been recovered from ixodid ticks, though the role of ticks in transmission of bartonellosis is not clear yet. It is useful to know that cat bites can also transmit other dangerous infections such as plague, tularemia, sporotrichosis,
nocardiosis and infections with *Pasteurella multocida* and *Capnocytophaga canimorsus*.

Bacteraemia with *B. henselae* can persist in cats for months (asymptomatic for the animal). A biopsy of the skin lesion or an affected lymph node can help to cement the diagnosis. Antibodies against *B. henselae* can be detected serologically. In lymphadenitis azithromycin for 5 days is first line treatment, alternatively clarithromycin, ciprofloxacin or doxycycline for 7-10 days can be used.

LAST UPDATED BY ADMIN ON JUNE 23RD, 2022

**Spirochaetal diseases**

**Summary**

- Spirochaetes are very thin, spiral shaped organisms.
- There are a number of species.
- The bacteria take their name from various sources: *Borrelia* (after the French bacteriologist Amédée Borrel), leptospires (meaning “fine coils”), treponemes (“turning, drilling”).
- Spirilla are usually classified separately.
- As yet there is no definitive nomenclature for the various subspecies.

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<thead>
<tr>
<th>Bacteria</th>
<th>Disease/Condition</th>
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<tbody>
<tr>
<td><em>T. pallidum</em></td>
<td>syphilis, bejel (non-venereal syphilis)</td>
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<tr>
<td><em>T. pertenue</em></td>
<td>framboesia (= yaws, = pian)</td>
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<tr>
<td><em>T. carateum</em></td>
<td>pinta</td>
</tr>
<tr>
<td><em>L. interrogans</em></td>
<td>Weil’s disease and more mild forms</td>
</tr>
<tr>
<td><em>B. recurrentis</em></td>
<td>louse-borne borreliosis</td>
</tr>
<tr>
<td><em>B. duttonii, B. hispanica, B. persica and others</em></td>
<td>tick-borne borreliosis</td>
</tr>
<tr>
<td><em>B. burgdorferi sl</em></td>
<td>Lyme disease</td>
</tr>
<tr>
<td><em>B. vincenti</em></td>
<td>tropical ulcer, Plaut-Vincent’s angina, cancrum oris, Fournier’s scrotal gangrene, trench mouth (necrotising ulcerative gingivitis)</td>
</tr>
</tbody>
</table>
Spirillum minus | sodoku or rat bite fever
Streptobacillus moniliformis | Haverhill fever

There are Treponema diseases:

1. Venereal syphilis or Lues
2. Non-venereal syphilis or Bejel
3. Framboesia or Yaws or Pian
4. Pinta

**Non-venereal treponematoses**

Treponematoses are diseases caused by treponemes. These are bacteria with a spiral structure ("trepo" = turn; "nema" = thread). They belong to the *Spirochaetaceae*. They cause 4 different chronic exclusively human diseases. There is **no animal reservoir**. The various treponemes cannot be cultured in vitro (*Treponema pallidum* can be cultured with some difficulty in tissue culture and in rabbit testicles). Morphologically **they cannot be distinguished one from another** and all give positive results on so called syphilis serology. They are all sensitive to penicillin. Prevention varies.
Geographical distribution of non-venereal treponematoses.

**Bejel or Njovera or Treponarid**

Bejel is caused by *Treponema endemicum* (*Treponema pallidum endemicum*). The disease occurs (occurred) in foci in sub-Saharan Africa, in the Middle East, central Australia and in Asia, in temperate to warm dry climates (e.g. Sahel area, Zimbabwe, Botswana). The disease formerly also occurred in Bosnia. Between 1950 and 1960 there were large-scale campaigns to control the disease in the Sahel countries. At present the disease has become rare.

Infection mainly results in **skin and skeletal abnormalities**. Transmission is not via sexual intercourse but through contact. The incubation time is unknown. As a rule, non-venereal or endemic syphilis occurs in childhood. The **oral mucosae are the most important source of infection**. Children are mainly infected by objects they use such as contaminated beakers (bacteria entering through the mouth). In this way they probably acquire immunity against *T. pallidum* before puberty.
and are protected against later venereal syphilis.

There is an **early stage** which lasts some 5 years. This is characterised by skin lesions and oral mucosal lesions which occur intermittently. **Osteitis and periostitis** can occur. In rare cases there are **delayed lesions (gummata)**. Gangosa is characterized by destruction of the nose, lip and palate and can lead to severe mutilation. Treatment consists of a single IM administration of 1.2 or 2.4 million units of long-acting benzathine penicillin. A single dose of azithromycin can also be used for treatment but some guidelines prefer to safeguard azithromycin as reserve antibiotic. Tetracyclines can be used as an alternative. Plastic reconstructive surgery is often needed to repair mutilations.

**Framboesia or Yaws or Pian**
Framboesia, yaws, pian. Infection with Treponema pallidum pertenue. Copyright ITM, photo by Dr Jef Van den Ende.
Framboesia, yaws, pian. Infection with Treponema pallidum pertenue, resulting in plantar hyperkeratosis with painful cracks and fissures. Copyright ITM, photo by Dr Jef Van den Ende.

Yaws is caused by *Treponema pertenue* or *Treponema pallidum pertenue*. The transmission of yaws in man through inoculation was demonstrated by Paulet in 1848 and by Charlouis in 1881, predating the discovery of *T. pertenue* by Castellani in two Ceylonese patients with the disease (called “parangi” there).

This treponematosis is transmitted from person to person via direct skin and mucous membrane contact (small scrapes). It is a disease of poor isolated rural communities in warm, humid, tropical areas of Africa, Central and South America, and some islands in Southeast Asia. There is hardly any congenital transmission. Framboesia has currently become rare and has been eliminated in some areas (e.g. in Esmeraldas, Ecuador) but may be re-emerging in some areas. This is explained by the deterioration in clinical medical care in certain areas (it is easy to diagnose and the treatment is cheap and simple) and the lack of large-scale treatment campaigns. *T. pertenue* can infect baboons, chimpanzees and some other monkeys, but the importance of this is not clear. It is unlikely that an animal reservoir plays an important epidemiological role as far as can be judged at this time.

**Clinical Aspects**

The skin and skeleton are affected, deep organs are always spared. The disease is characterised by wart-like skin lesions with the appearance of strawberries (hence the name; yaw = strawberry). The skin lesions return periodically.

The primary lesion is extragenital. It may consist of one warty lesion but sometimes there is an initial parent lesion with various satellite lesions. In most cases the lymph nodes are swollen. If the hypertrophic, papillomatous epidermis is removed an exudate with a crust forms. There is no deep ulceration. These early lesions heal without leaving scars. After healing some residual skin discoloration may remain.

A few weeks to months after the primary lesion, more scattered secondary macular or papillomatous lesions occur. The early skin lesions which contain a great number of treponemes, tend to be multiple and moist. They occur in flare-ups which last weeks or months in each case. Without treatment this can last 3 to 5 years. When there is a flare-up, there can be general malaise together with joint pain and fever. The skin lesions may persist for 3-6 months. On the palms of the hand and the soles of the feet the skin can thicken, become hyperkeratotic and itchy and painful fissures appear. These result
in the characteristic gait, the so-called “crab gait”. A severe infection with *Tunga penetrans* (sand fleas) can sometimes produce a similar picture, but on closer inspection the difference is clear. Sometimes there is involvement of the skeleton. Chronic inflammation of the bones of the fingers (dactylitis) should be distinguished from the more acute dactylitis seen in sickle cell anaemia. Since the general availability of penicillin occasionally mild forms of yaws are seen with only one or just a few small lesions, a few papules or limited hyperkeratosis. It is not known whether the pathogen has a reduced sensitivity to penicillin.

Late-onset framboesia occurs in 10% of patients (after > 5 years). Characteristic of this condition are **sporadic gummata in the skin**: deep crater-like ulcers which later heal with the formation of scars covered by a thin skin. Treponemes are very rare here and the lesions are therefore not particularly infectious. Contracture of the affected limb may occur. Joints may stiffen and chronic osteitis and periostitis can lead to bent legs (sabre tibiae).

**A number of secondary lesions occur in framboesia:**

- **Nodules**: mainly around joints. Hard nodules which are loose from the skin and the deep tissue on the extensor side of elbows, wrists also on trochanters, ankles and sacrum. The aetiology is unclear and a differential diagnosis has to be made with onchocerciasis.

- **Gangosa**: this is rapid tissue loss from the nose, palate and upper lip, caused by a gumma in this area. To be differentiated from espundia (mucocutaneous leishmaniasis), deep mycosis (e.g. blastomycosis), leprosy and noma (= cancrum oris associated with among other things, malnutrition caused by infection with *Borrelia sp.* and fusobacteria).

- **Goundou**: swelling of the nose and upper jaw bones due to inflammation of the bones of the nose (osteitis). The rare fungal infection rhinoentomophthoromycosis can sometimes be confused with this.

- **Gumma**: a subcutaneous gumma can manifest itself as a cold abscess.
Framboesia, yaws, pian. Infection with Treponema pallidum pertenue. Deformed tibia, the so-called sabre tibia.
Melorheostosis can resemble Treponema pertenue sequellae, such as sabre tibiae. The radiological lesions often look like dripping candle wax. Copyright ITM
Framboesia, yaws, pían. Infection with Treponema pallidum pertenue. Notice the deformed tibiae, the so-called sabre tibiae. Copyright ITM, photo by Dr Jef Van den Ende
Framboesia, infection with Treponema pertenue. The name gangosa refers to the ulcerative destruction of the centre of the face. If a child survives noma, similar lesions can be found in adults.

**Treatment**

In patients over 10 years of age, a **single IM injection of 2.4 million units of benzathine penicillin** or a **single dose of azithromycin 30 mg/kg (max 2 gr)** is sufficient. Half the dose of penicillin should be used in younger children. In the early stages this produces fairly spectacular results. All individuals who have been in contact with the patient should also be treated. Doxycycline can be used for one week as an alternative. Erythromycin is less active. Azithromycin has been successfully used in mass treatment programs to enable yaws elimination. In certain areas the eradication of framboesia has been followed by an increase in venereal syphilis.

After successful treatment titers of nontreponemal serological tests become negative within less than 2 years.

**Pinta**
Pinta, depigmented skin lesions. Infection with Treponema carateum. Photo Cochabamba, Bolivia

Pinta is caused by *Treponema carateum*. This treponematosis is limited to a few foci in Central America, Colombia and southern Mexico. Cases of pinta are becoming less and less frequent. Only the skin is affected. Transmission is through contact. The primary lesion is a scaling papule which is often itchy. This appears within ten days after exposure. The papule increases in size over the following 2 to 3 months and forms a flat, scaly plaque. There is no latency period, unlike other treponematoses. A few months to more than one year later, a mild itchy maculopapular rash develops. The spots are distributed randomly over the whole of the body. They have abnormal changing pigmentation: initially blue to purplish then brown. They still contain treponemes. Later the lesions become atrophic and fade. After treatment with penicillin the lesions remain discoloured. The main problem is cosmetic, to be distinguished from other causes of hypopigmentation such as vitiligo and leprosy. There are no ulcers and no bone lesions. Pinta does not protect against the other treponematoses.

Summary

<table>
<thead>
<tr>
<th></th>
<th>Syphilis</th>
<th>Bejel</th>
<th>Yaws</th>
<th>Pinta</th>
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<tr>
<td><strong>Visceral lesions</strong></td>
<td>yes</td>
<td>no</td>
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</table>

Leptospirosis

**Summary**

- Leptospirosis: bacterial zoonosis
- Transmission via contact with contaminated freshwater
- Fever, muscle pain, cough, red eyes
- Hepatomegaly, icterus, hemorrhagic tendency, meningitis, nephritis
- Difficult clinical diagnosis: water contact, leukocytosis, urine analysis, lumbar puncture
- Serology and direct detection of bacteria are difficult to carry out
- Treatment tetracyclines, penicillin

**General**

Leptospirosis is the most widespread zoonosis worldwide, caused by the spirochetes of the genus *Leptospira*. An estimated one million people are infected annually, with 60,000 deaths. It is most prevalent in tropical regions, but there are occasional cases in Belgium and the Netherlands. Leptospires are the only pathogenic spirochaetes that are **free-living in the environment**. In comparison, *Treponema pallidum* is only found in humans, and *Borrelia* spirochaetes are only found in arthropods and mammals.

The severe form of leptospirosis was described in 1886 by the German Adolf Weil, Professor of Medicine at the University of Heidelberg. It is therefore still called **Weil’s disease**. In 1907 Stimson discovered the organism in kidney tissue from a patient who died during a yellow fever epidemic (see Clinical aspects).

Clinically it is indeed **tough** to differentiate between yellow fever and leptospirosis. In regions where scrub typhus and hantavirosis are endemic, differentiation between *Orientia tsutsugamushi*, hantavirus infection and leptospirosis on clinical criteria alone is impossible.

**Taxonomy**

The bacteria are very delicate and spiral-shaped. They have a typical terminal hook (Gr. leptos = delicate, slender, speira = spiral, interrogans = question mark). The bacteria are so thin that they cannot be detected with normal light microscopy. They can be seen using phase contrast or dark-field microscopy (urine) and using silver staining of tissue sections. Leptospires have a characteristic **double membrane architecture with features of both Gram-positive and Gram-negative bacteria**. Traditionally, the genus *Leptospira* contained two species: *Leptospira interrogans* sensu lato, which was pathogenic and *L. biflexa* sensu lato which was non-pathogenic for man. However, the taxonomy of *Leptospira* has undergone significant changes due to large-scale whole-genome sequencing. There are currently 64 species, split into two clades (pathogenic ‘P’ and saprophytic ‘S’) and four subclades. 17 Pathogenic species are classified in subclade P1 (*L. mayottensis, L. alexanderi, L. kirschneri, L. kmetryi, L. alstonii, L. adleri, L. barantonii, L. ellisii, L. dzianensis, L. gomenensis, L. putramalaysiae, L. tipperaryensis, L. borgpetersenii, L. interrogans, L. noguchii, L. santarosai, L.

**Transmission**

The pathogenic bacteria can **survive in freshwater** but die in seawater. Infected animals retain **bacteria in their kidneys for a long time and eliminate them in the urine**. Transmission follows contact with fresh water contaminated with the urine of infected animals. **Rats** form the main reservoir, but other animals, such as cattle, dogs, cats and pigs may also become infected.

Leptospires are killed by gastric acid and bile salts. They penetrate the body via wounds and the mucosa of the mouth, nose and eyes (conjunctivae). **Water** is the most important transmission route, but direct contact with infected animals may also be significant (slaughterhouse workers, veterinary surgeons). It is a disease associated with **certain occupations**, e.g. workers in paddy fields or on sugar cane plantations, farmers, workers in sewers and canals, gold prospectors (gold dust obtained from water courses). People who bathe or swim in infected surface water are at increased risk of this zoonosis. Now that rafting, kayaking and adventure sports in tropical regions have become popular, there is an increase in leptospirosis in tourists. Ideal conditions for transmission are produced when dirty streets with large rat populations are flooded.

Heavy rainfall or flooding in endemic areas can lead to large outbreaks of leptospirosis, especially in areas with poor housing and sanitation. Outbreaks have also been reported in triathlon participants where the swimming was in fresh water.

**Clinical aspects**

Given the many species of leptospires, a **broad spectrum of diseases is possible**. Symptoms
Bacteria | 195

range from mild fever with a ‘flu’-like syndrome to atypical pneumonia, myocarditis, aseptic meningitis or the severe Weil’s disease with liver and kidney failure, meningitis and hemorrhage. The disease course has three phases: the first septicaemic, the second with leptospiruria (leptospires in the urine) and the third convalescence phase.

During the first phase, the leptospires are present in the blood in low numbers (too low to be detected in a blood smear using phase contrast microscopy). Subsequently, the organisms disappear from the blood due to the formation of antibodies. The cellular defense also clears the bacteria from the various tissues. Leptospires persist in the kidney. In the renal tubules, the organisms can multiply and cause renal damage. Bacteria are eliminated with the urine, although the concentration is quite low: \(< 10^4/ml\) urine. The bacteria may remain in the kidneys for months, even after clinical recovery. Leptospires might also persist in the choroid plexus of the brain.

Most cases of leptospirosis are mild and self-limiting or asymptomatic. Mild forms are often atypical and are generally missed unless they are specifically sought for. The acute phase of leptospirosis usually starts 5 to 14 days after exposure (maximal incubation range 2 to 30 days). Fever, rigors, myalgias (mainly in the calves and lower back), headache and general malaise usually last two to nine days. Patients can sometimes pinpoint within the hour when the illness began. Next, the fever may subside for a few days and then increase once more (biphasic fever) during the “immune” phase. The absence of this fever pattern does not rule out the disease. Significant muscle pain is almost always present. If it is absent, the diagnosis is improbable. There is sometimes a sore throat and a dry cough, later possibly hemoptysis. In 10 to 30% of patients, the lower legs have a spotty skin rash. [This was initially described as “Fort Bragg Fever” caused by \(L.\) \textit{interrogans autumnalis}]. The eyes are often bloodshot due to dilation of conjunctival blood vessels causing conjunctival erythema. Pus discharge is absent, unlike in purulent conjunctivitis. Subconjunctival hemorrhage can occur on top of the conjunctival suffusion. During the immune phase, anterior uveitis presenting as acute onset pain and redness of the eye(s) may occur. Posterior uveitis (chorioretinitis) is less common and presents with decreased vision or floaters. Two-thirds of patients suffer nausea and/or vomiting. Swollen lymph nodes are only present in a minority of patients. The spleen is swollen in 20% of cases.

Marked elevation of CK levels indicates muscle damage and occurs only in severe cases. Muscle pain, predominantly in the calves, can lead to local swelling, which can be so severe that patients cannot walk anymore. Pectoral, back and abdominal muscles may also be involved. Palpation of the calves tends to be painful. The injured muscles heal without scarring. CK levels and muscle symptoms usually diminish in the second week of illness. Rhabdomyolysis seems to be secondary to direct muscle cell invasion with cell necrosis and small intramuscular hemorrhages. Myocarditis occurs and often leads to congestive heart failure and cardiogenic shock. Electrocardiographic abnormalities are
Weil’s disease is a syndrome characterized by icteric leptospirosis with fever, jaundice and renal failure. Lung bleeding with ARDS, myocarditis and rhabdomyolysis may accompany this syndrome. Involvement of the liver is characterized by hepatomegaly, jaundice and a hemorrhagic tendency. Scleral icterus and jaundice are accompanied by a marked conjugated bilirubin elevation with normal or slightly elevated aminotransferases. The gall bladder may become inflamed (acute cholecystitis). Liver failure is rare.

Atypical pneumonia with possible blood-tinged sputum can be expected in severe cases. Pulmonary lesions are primarily hemorrhagic rather than inflammatory. Patients are at risk for secondary bacterial pneumonia.

Kidney damage leads to proteinuria, hematuria and uremia. Hypovolaemia and poor renal circulation may further exacerbate renal damage. Hypovolaemia is characterized by oliguria, low blood pressure, diminished skin turgor and flat neck veins. If it is not corrected by giving fluids, tubular necrosis will follow. Temporarily hemodialysis is needed in severe renal failure. Sterile pyuria, proteinuria, granular casts, myoglobinuria and enlarged kidneys occur in some patients. The haem part of myoglobin separates form the globin moiety in an acid environment (pH < 5.4). Renal tubular obstruction due to the precipitation of myoglobin is dangerous. Myoglobin is less toxic if there is no dehydration or acidosis. Therefore alkalinization of the urine is essential.

Meningism may occur early but is more frequent in the immune phase. Neck stiffness is present in half the patients with aseptic meningitis. The CSF typically has a neutrophilic or lymphocytic pleocytosis with mild proteinorachia. CSF pleocytosis may last for up to three months. Meningitis is attributed to the immune response rather than a true CNS infection. However, recent studies could Leptospira in the CSF by polymerase chain reaction.

In severe leptospirosis, the total period of illness is approximately three weeks to one month. The mortality is between 5 and 30%; severe icterus has a poor prognosis. If the patient survives, there is usually no residual damage. A long convalescent period is typical.

Differential diagnosis:

This is very broad because of the variable symptoms. It includes Hantavirus infection, influenza, gastro-enteritis, meningitis, malaria, hepatitis, cholangitis, rickettsiosis (e.g. scrub typhus), borreliosis, typhoid fever, Reye’s syndrome, arboviroses such as yellow fever, Rift valley Fever, Crimean-Congo
hemorrhagic fever and West Nile fever as well as arenaviroses. In the case of hemorrhagic tendency, Gram-negative bloodstream infections and various viral hemorrhagic fevers should be considered.

**Diagnosis**

Confirming leptospirosis is quite difficult, and the disease is often missed. The disease should be clinically suspected in patients exposed to endemic or outbreak settings who present with systemic febrile illness without an alternative explanation. Exposure to potentially contaminated water (occupation, accident, swimming, recent travel to flooded areas etc.) and rat exposure should be enquired. Aseptic meningitis, uveitis, jaundice, acute febrile kidney injury, pulmonary hemorrhage and conjunctival suffusion should raise the suspicion for leptospirosis.

Microscopy of Leptospira sp., bacteria that cause leptospirosis. Photo Cochabamba, Bolivia

There is proteinuria, pyuria and microscopic haematuria. The cerebrospinal fluid initially contains
neutrophils. Later, lymphocytes predominate, together with elevated protein and normal glucose. In general, there is significant leukocytosis, but this is not constant. Thrombocytopenia is common. Early in the disease, leptospires can rarely be found in the blood, urine or cerebrospinal fluid (the tests are not very sensitive). Subsequently, the bacteria are only found in the urine. Since these are very thin organisms (0.1 µm diameter), a dark-field microscope is needed to detect them in a blood smear. Indirect illumination is used in this method instead of direct illumination so that fine structures can be detected which are not visible with the traditional microscope. This method is not very sensitive and has been responsible for many errors (many false positives and false negatives).

**Serology** can be performed. The traditional serology using micro-agglutination test or MAT requires a well-functioning laboratory, which will not be available in practice in low resources settings. A single positive or negative IgM or IgG cannot confirm or rule out infection, even though a single IgG titer (>1:800 on MAT) strongly supports infection. A second sample 7 to 14 days after the first antibody test should be obtained, and a four-fold increase in IgG titer confirms infection. Antibody tests that do not detect all serovars may produce false negative results. The interpretation of MAT serology results to identify the responsible serovars is rather difficult because the highest titer does not necessarily correlate with the actual serovar responsible for the infection.

**Culture** of the bacteria is the gold standard but is **not practical in most settings**. The culture of leptospires is complex and requires non-standard equipment. Special media such as Fletcher’s, Ellinghausen’s, or polysorbate 80 media are required for isolation. Blood and CSF specimens are positive during the first ten days of the illness. Urine cultures become positive during the second week of illness and remain so for up to 30 days after the resolution of symptoms.

In high-resource settings, **PCR** is used on blood samples, urine, CSF and tissue biopsies. Whereas PCR detects leptospires during the first week of symptoms, urine samples are particularly valuable beyond the first week of illness. The sensitivity of PCR ranges from 40 to 60 percent in blood samples; the specificity exceeds 95 percent.

Antigen detection using a monoclonal antibody-based direct ELISA (anti-LipL32 antibodies) on blood has shown promising results in Sri Lanka but needs validation in larger international studies.

**Treatment**

Most patients with leptospirosis will recover without antibiotics. Although robust evidence is lacking, **early treatment initiation** might prevent evolution to severe disease. Antibiotics such as tetracyclines within the first 4 days are assumed to shorten the illness. Sometimes leptospires persist
Bacteria | 199

in urine, despite the correct treatment. Oral doxycycline 200 mg per day for 1 week is the preferred regimen for mild infections. If there is vomiting, IV penicillin is used. For severe infections, ceftriaxone can also be used. This allows for once-daily dosing, which is more practical than the multiple dosing schemes using penicillin. Azithromycin and ampicillin are also active against leptospires. Chloramphenicol is not. Most patients with critical illness will have been placed on an empirical antibiotic treatment. However, since the pathophysiology suggest an exaggerated immune reaction, the beneficial effect in severe disease remains controversial.

The immune-triggered second phase suggests a role for corticoids in Weil’s disease. Some studies suggest a possible benefit, but more studies are needed.

Symptomatic and supportive therapy is vital. If there is myoglobinemia, alkalinization of the urine is essential to limit renal damage. In severe disease, hemodialysis, mechanical ventilation and blood products can be life-saving.

**Prevention**

Since rats form the main reservoir and contaminate surface water and drains, their control is important for prevention. Nevertheless, it should not be forgotten that the animal reservoir is much broader (e.g. dogs etc.) and cannot be eradicated completely. Avoiding sources of infections, such as water contaminated with animal urine, is advised. Wearing boots when working in stagnant water is advisable. Chemoprophylaxis of 200 mg doxycycline per week may be taken as a preventative in high-risk situations like flooding in an endemic region. After infection, there is protection against the infecting serovar but no cross-immunity.

Human vaccines have been developed, but they are serovar-specific, and none of them is widely available. Animal vaccination can provide variable levels of protection for animals and humans.

**Borreliosis**

**Relapsing fever**
Summary

- Spiral shaped bacteria, transmitted by ticks (endemic) or lice (epidemic)
- Recurrent fever, rash, hepatosplenomegaly, red eyes, haemorrhagic diathesis, muscular pain, coughing, confusion, neurological complications
- Thick film test positive, esp. in beginning of attack
- Treatment with penicillin or tetracyclines (e.g. doxycycline)

General

*Borrelia* sp. are very thin, spiral shaped bacteria. They are larger, longer and have looser coils than treponemes or leptospires. They are responsible for major diseases, including recurrent or relapsing fever. In 1868 the German Otto Obermeier identified the microorganisms during an epidemic in Berlin. The pathogenic potential was demonstrated in 1874 by Gregor Münch, who inoculated himself with *Borrelia recurrentis* and survived the subsequent relapsing fever. The French microbiologists Sergent and Foley identified the body louse as the vector. The British pathologist Joseph Dutton (famous because of *B. duttoni*) discovered an alternative vector: the Argasid soft tick Ornithodoros moubata. He injured himself while performing an autopsy on a patient who had died from borreliosis and died himself from relapsing fever. During his research into East Coast fever in East Africa, Robert Koch discovered that transovarial transmission took place in these ticks. Charles Nicolle and co-workers established that *Borrelia recurrentis* disappeared from the intestine of the louse 24 hours after a blood-meal, to appear again suddenly in the haemolymph of the insect after 6-8 days. Experimental animals such as rats and mice can be inoculated successfully. *Borrelia recurrentis* can be grown in chicken embryos and since 1994 in-vitro.

There are two types of borreliosis: relapsing fever, louse-borne borreliosis (*Borrelia recurrentis*) and tick-borne borreliosis (*Borrelia duttoni* and many other varieties, depending on the geographical region). The bacteria are morphologically identical. The name “tick-borne borreliosis” sometimes causes confusion, as *Borrelia burgdorferi* is also transmitted by ticks, but this organism does not cause relapsing fever.

Epidemic, louse-borne relapsing fever

In the epidemic form of borreliosis the bacterium *Borrelia recurrentis* is transmitted by lice. The vector is the common body louse (*Pediculus humanus corporis*). [The body louse is also the vector of epidemic typhus and of *Bartonella quintana*. This insect is not to be confused with the pubic louse (*Phthirus pubis*).] The head louse (*P. h. capitis*) hardly ever plays a part in transmission. There is no
transovarial transmission of *Borrelia recurrentis* in the louse. **Humans are the reservoir** of the disease.

The louse is infected by sucking blood at the time the patient has an outbreak of fever. At this time the levels of bacteria in the blood are at their highest. The bacteria penetrate the insect’s intestine and multiply in the haemolymph ["blood"] of the louse. The bacteria do not penetrate the salivary glands. The disease is not transmitted by the bite itself. **If an infected louse is crushed on the skin when scratching, the bacteria can penetrate into the skin.** Lice do not like high temperatures and will readily leave a person who has a fever. In the event of poor hygiene and close physical contact between people lice can pass from a sick person to a healthy person.

The disease is **rare but can occur all over the world.** The geographical distribution of LBRF has declined due to improvements in living standards. Currently the disease is primarily found in limited endemic foci in Ethiopia but also in Somalia and Sudan. The disease has also been recorded in the rural Andean community in Peru and in northern China. Epidemics occur in conditions of poor hygiene, overcrowding and malnutrition, such as in floods, mass migration, earthquakes, concentration camps and refugee camps, war, and in the slum districts of large towns. Body lice multiply rapidly and a population can increase by 11% per day. Infection is more frequent in the cold months. People live closer together then, wear more clothes, so there are more lice and consequently more transmission. **Mortality can be very high (30 to 80%).** Between 1910 and 1945 there were 7 large epidemics in Africa, Eastern Europe and Russia with 15 million cases and 5 million dead.

**Endemic, tick-borne relapsing fever**

This is a sporadic, endemic disease in a number of areas caused by *Borrelia duttoni* and related bacteria. The vectors are **soft ticks** (*Ornithodoros sp.*). In West Africa *O. erraticus* is responsible for the transmission of *B. hispanica*. In Central, Eastern and Southern Africa *Ornithodoros moubata* is the main vector (*B. duttoni*). These latter ticks infect people through their saliva and through coxal fluid. It is mainly an infection of rodents. These animals are the principal reservoir. Because the bacterium in ticks passes from one generation to the next by transovarial transmission, the ticks themselves also form a reservoir. People can be infected by ticks for example when walking through grass or bushes. In Central Africa there is a domestic variety whereby the ticks live in cracks in the walls of mud huts and are therefore more likely to bite humans. The people who are infected are then the main reservoir. Ticks can live for a number of years (exceptionally up to 15 years) unlike lice (a maximum of 2 months). They can survive for a long time without a blood-meal. **Mortality in man is lower with tick-borne borreliosis (2 to 5%) than with the epidemic form.** The local population builds up immunity from repeated infections; they usually have a mild form. The bacteria can cross the
placenta to the fetus.

Over the course of an infection in a single human host *Borrelia sp.* regularly display antigenic variation, mainly by changing various surface proteins (“variable large proteins and variable small proteins”).

**Clinical Aspects**

After an incubation period of **4 to 14 days** (**1 week on average**), the patient suddenly develops a violent fever (39° to 41°C). This is accompanied by a high bacteraemia: $10^{6-8}$/ml. The concentration of bacteria is so high that they can be detected with the **thick film test or a thin blood smear** (in classical Gram-negative bacteraemia (e.g. *E. coli*) the concentration of bacteria is much lower). The patient suffers from headache, muscular pain and pain in the joints. There is often a dry cough and dyspnoea, which can be quite severe. The patient sometimes suffers from abdominal pain and diarrhoea. The patient is frequently jaundiced. The spleen, the liver and the lymph nodes are often swollen. Neurological abnormalities occur. The conjunctivae are often red. Sometimes (in 4 to 50% of cases) there is a discrete rash which usually appears when the first fever peak subsides.

Diffuse intravascular coagulation (DIC) and thrombocytopenia, petechiae and haemorrhaging can occur, e.g. epistaxis (nose bleeds). Sometimes (1/3) a considerable leucocytosis can be present, but leukopenia can also occur. The cerebrospinal fluid can contain an increased number of lymphocytes (mainly in endemic tick-borne borreliosis). The fever suddenly disappears after 2 to 8 days on average 5 days. This is usually accompanied by an aggravation of the symptoms, hypotension and sometimes death. The prognosis is worse with louse-borne borreliosis, when there is manifest jaundice, hypotension and high bacteraemia (which can be objectivised in a thin blood smear). There is high neonatal mortality (50%).

The first febrile episode is followed by a period of **3 to 30 days** (**on average 9 days**) without fever. In 60% of patients this is followed by a second febrile period, which is somewhat less severe than the first and also lasts for a shorter time (on average 2 days). This can be repeated a number of times: maximum 4 times in case of louse-borne borreliosis, maximum 11 times in case of tick-borne borreliosis. This characteristic explains why it is called “relapsing fever”.

Complications are meningo-encephalitis with as sequelae facial paralysis, deafness and paralysis of the eye muscles (mainly endemic tick-borne borreliosis). Most spirochaetes are neurotropic. Myocarditis and abortion may also occur. If a pregnant woman has relapsing fever she has around a 50% risk of going into labour.
Diagnosis

The clinical **signs and symptoms are not specific** apart from the **recurrent bouts of fever**. At the beginning of a febrile episode bacteria are found in the blood. These very thin spiral shaped bacteria (0.5µm) can be seen in an unstained unfixed preparation because of their typical mobility. They can also be stained with Giemsa and Wright stain. Staining with Diff-Quik (xanthene thiazine stain) is an alternative. They are found between the red blood cells. The fact that the bacteria can be seen in peripheral blood is explained by the very high density of the bacteria. *Borrelia* spp can be cultured through animal inoculation or in vitro cultivation in a Barbour-Stoenner-Kelly (BSK) medium. PCR and serology are only available in a few reference laboratories.

The differential diagnosis includes **many febrile conditions** including malaria, typhoid fever, hepatitis, amoebic hepatic abscess, leptospirosis, rat bite fever, septicaemia, arbovirosis, ehrlichiosis and anaplasmosis, babesiosis, rickettsial diseases (can also be transmitted by lice and ticks).

Treatment

**Tetracyclines** are the first choice, e.g. doxycycline. A single administration is often sufficient. Alternatively erythromycin can be given. In the case of louse-borne borreliosis, in ± 90% of patients a spectacular deterioration in the symptoms is seen 1 to 3 hours after starting therapy: headache and muscular pain, tremor, very high fever, tachypnoea, tachycardia and initial hypertension. This is followed shortly after by excessive perspiration and hypotension and sometimes shock. This is a so-called **“Jarisch-Herxheimer”** reaction which usually lasts 6 to 12 hours. This reaction rarely occurs (1%) with tick-borne borreliosis. The reaction was first described in syphilis patients who were being treated with mercury chloride or penicillin. It can also occur when treating other infections caused by intracellular bacteria (such as Brucella, Q fever). It has a mortality rate of about 5%. It is thought that it develops from various substances being released from the destroyed bacteria, together with high concentrations of certain cytokines (e.g. TNF alpha, IL-6 and IL-8). Steroids are not effective in preventing the reaction. It has been shown that treatment with anti-tumour necrosis-alpha antibodies mitigates the Herxheimer reaction. The patient must be kept under close supervision (bed rest, IV infusion). Penicillin is less frequently associated with Herxheimer reactions but is less effective (often further recurrences).

Prevention

There is no vaccination and no lasting immunity after a patient has had the infection. In the case of an epidemic (louse-borne borreliosis) mass delousing is often carried out (2 x with an interval of 2 weeks) for example in refugee camps. This is based on the use of insecticides and hot sterilisation (boiling
Bacteria and washing) of clothes.

**Borrelia vincenti**

It is not clear whether this bacterium is itself a pathogen or whether it is present as a saprophyte in necrotic material. The bacteria can, unlike the other *Borrelia* be cultured in an anaerobic environment. In combination with certain anaerobic bacteria (fusobacteria = anaerobic Gram-negative “fusiform bacteria”) this bacterium is suspected of causing ulcerative damage in the:

- **throat**: Plaut-Vincent’s angina. This results in a major throat infection with localised necrosis. DDx: diphtheria of the throat, local anthrax or plague.
- **gums**: Trench mouth or Vincent’s stomatitis, a necrotising and ulcerative gingivitis of the cheek. This occurs in malnourished children and sometimes after herpes simplex.
- **cheeks / lips**: Cancrum oris (noma) is characterised by pain and extensive tissue destruction. Treatment consists of penicillin, correct nutrition and treatment of any underlying disorder (e.g. kala-azar, etc). Plastic surgery will be needed.
- **scrotum**: Gangrene of the scrotum (Fournier’s gangrene).
- **skin**: Painful (in the acute stage), purulent, foul-smelling ulcers, mainly on the legs or feet (phagedenic or tropical ulcer). Ulcers such as this can drag on for years or sometimes heal spontaneously. In some patients a spinocellular carcinoma develops which is invasive locally and can metastasise to the local lymph nodes. Treatment consists of penicillin and metronidazole. Local wound cleaning, antiseptics and non-adhesive dressings are important. Dry dressings should be avoided because they prevent the forming of new epithelium (when the dressing is removed the new cells are pulled off).

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**Rat Bite Fever**

**Summary**

- Infection by bacteria: *Streptobacillus moniliformis* or *Spirillum minus*
- Rat bite fever is named sodoku in Asia, caused by *S. minus*
- Haverhill fever is rat bite fever caused by *Streptobacillus moniliformis* after ingestion of food or water contaminated with rat faeces
Rat bite wound followed by fever, lymphadenopathy, migrating arthralgia, skin rash and muscle pain
- If transmitted via infected drink: episodic fever, throat pain, rash, muscle and joint pain
- Systemic complications possible: myocarditis, pneumonia, abscesses, meningitis
- Treatment with penicillin

**General**

Rat bites may give rise to infection with various bacteria but two deserve special attention. *Spirillum minus* is a systemic zoonosis occurring mainly in Asia. Rat bite fever caused by *Streptobacillus moniliformis* has a more cosmopolitan distribution and is mainly recognised in Europe and North America. A third species causing rat bite fever – *Streptobacillus notomytis* – has only been reported rarely. Infection in third world countries will probably be discovered as soon as better diagnostic facilities are available. Rat bite fever may trigger intermittent fever which may make it similar to other infections.

**Clinical aspects**

*Spirillum minus* is a small spiral-shaped bacterium and is usually classified as a spirochaete and is unable to be cultured. The bacterium has flagellae and moves quickly unlike *Streptobacillus moniliformis*.

*Streptobacillus moniliformis* is a difficult to culture pleomorphic non-motile Gram-negative rod-shaped bacterium. Its name refers to the necklace like morphology exhibited by the bacteria that form thin branched filaments.

The disease caused by *S. minus* is known as sodoku in Asia (a Japanese name: *so*: rat, *doku*: poison). Infection may follow a rat bite or the consumption of water or milk contaminated by rat urine or faeces. *Streptobacillus moniliformis* infection occurs after ingestion of food or water contaminated with infected rat faeces. The disease is known as **Haverhill Fever**. The name Haverhill refers to a small town in Massachusetts where an epidemic broke out in 1926 following the consumption of contaminated unpasteurized milk. The bacteria occur naturally in the nasopharynx of rats and are found in 50 to 100% of rats living in the wild. The risk of rat bite fever due to *S. moniliformis* after a rat bite is estimated to be 10%. Not only rats, but also other rodents such as mice, gerbils, squirrels or carnivores or omnivores which eat rodents (cats, dogs, pigs, weasels, ferrets) can transmit the bacteria. People who work with animals (laboratory staff, some biologists) are at increased risk.
The incubation time is 1 to 30 days, usually approximately 1 week. If infection (S. minus and S. moniliformis) is transmitted orally, there are no skin wounds. A bite wound of S. minus causes local inflammation and even tissue necrosis with enlarged regional lymph nodes and its initial wound may reappear at the onset of systemic illness. Streptobacillus moniliformis bite wounds heal spontaneously.

After the wound has healed, intermittent chills, extreme fatigue, vomiting, diffuse muscle and joint pain and headache follow. Arthritis is not common in S. minus infection. S. moniliformis infection may give rise to an asymmetrical non-purulent poly-arthritis in up to 50% of patients. Generally the large joints are affected, such as the knees, ankles, elbows, wrists, shoulders and hips. Purulent arthritis is rare. If a patient is bitten on a finger, a neighbouring interphalangeal joint may exhibit impaired function.

Approximately two to four days after the beginning of the fever a skin rash occurs. This may have a morbilliform, pustular or petechial character. The rash is most pronounced on the hands and feet. Desquamation may occur. Somewhat later the patient develops painful pharyngitis. After an average of five days spontaneous improvement is seen. The fever disappears and the other lesions improve over the course of a few weeks.

After an irregular period of time there might be a relapse which resembles a picture of fever of unknown origin. This recurrence may persist for two years.

Complications include ulcerative endocarditis, subacute myocarditis, pericarditis, meningitis, pneumonia, amnionitis and anaemia. Abscesses may occur in any organ. In epidemics the name erythema artriticum epidemicum is used.

**Differential diagnosis:**

Differential diagnosis includes coxsackievirus (hand-foot-mouth syndrom) or an aspecific viral exanthema, meningococcal septicaemia, leptospirosis, erythema multiforme, secondary syphilis, rickettsiosis (RMSF [Rocky Mountain spotted fever]), tularaemia, Bartonella henselae (cat scratch disease) and infections which typically occur after bites, such as Capnocytophaga canimorsus, Eikenella corrodens or Pasteurella multocida infections. If joint problems are prominent, Lyme disease, acute rheumatic fever, brucellosis, gonococcal infection, septic arthritis, infectious endocarditis and auto-immune disorders may have to be excluded.

**Diagnosis**

A diagnosis may be reached clinically: unexplained (relapsing) fever or sepsis, maculopapular rash
and/or polyarthritis in patient with rat exposure. But even if there has been a rat bite, this will not always be reported when taking the history. Nevertheless this detail will be an important guiding factor. Some patients have a normal blood count, while others have significant leukocytosis (to 30,000) with left shift. Confirming a diagnosis microbiologically is extremely difficult: \textit{Spirillum minus} can be demonstrated using dark-field microscopy of a little fluid from the site of the bite but cannot be cultured yet in vitro. \textit{Streptobacillus moniliformis} can be cultured on specially enriched anaerobic media.

Serology (ELISA) may be carried out in specialised laboratories.

**Treatment**

Empirical therapy should be started instantly if rat bite fever is suspected since mortality may reach 13\% in untreated patients and laboratory confirmation is strenuous and time consuming. The treatment is based on penicillin (or a tetracycline in patients allergic to penicillin) preferably given for 14 days. There may be a Jarisch-Herxheimer-like reaction at the beginning of treatment. Ceftriaxone is also effective.

**Trachoma**

**Summary**

- Trachoma: important cause of blindness
- Chronic follicular keratoconjunctivitis caused by serotype A, B, Ba and C of \textit{Chlamydia trachomatis}
- Inflammation of the upper eyelid, followed by pannus of the cornea, entropion and trichiasis
- Treatment by tetracyclines or azithromycin
- Prevention by better hygiene, water, soap and fly control

**General**
The three most important diseases which lead to blindness in the tropics are onchocerciasis, vitamin A deficiency and trachoma. Other frequent causes are trauma, diabetes, leprosy, cataract, macular degeneration and chorioretinitis. The name trachoma refers to the raw appearance of the eyelid (Gr. “trachoma” = rawness). The term was first used by the Greek Pedanius Dioscorides (AD 50-70). Trachoma is a chronic form of conjunctivitis which is caused by some serotypes of *Chlamydia trachomatis*. Repeated reinfections are probably important in the ultimate pathology. The infection is characterised by progressive exacerbations and remissions, with follicular hyperplasia, corneal neovascularisation and scarring of the conjunctivae, cornea and eyelids. The disease occurs predominantly in dry areas of Africa (except for Congo), the Middle East, India and Southeast Asia. The disease is rare in the New World. The lack of water and soap for elementary hygiene plays an important role in transmission. Transmission takes place by hand-to-eye contact. Even sharing
infected utensils can lead to transmission. The role of flies (*Musca* sp.) was underlined by Jones, who showed that fluorescein-labelled eye secretions can be transmitted from child-to-child by these insects.

**Chlamydia trachomatis**

Chlamydiae are very small bacteria which have to live intracellularly. They were originally considered to be viruses, but it is now known that they contain both DNA and RNA and are structurally related to Gram-negative bacteria. Several species are known in the genus Chlamydia: *C. psittaci*, the pathogen of psittacosis; *C. pneumoniae* (old name TWAR), which provokes atypical pneumonia; and *C. trachomatis*, which has many serotypes. Serotypes A, B, Ba, and C cause trachoma. Serotypes D to K cause inclusion conjunctivitis in the newborn (“paratrachoma”), Reiter’s syndrome, non-gonococcal urethritis, epididymitis, cervicitis and P.I.D. (pelvic inflammatory disease). Neonatal conjunctivitis and pneumonia can be caused in the newborn by these bacteria. Serotypes L₁ and L₂ cause the sexually-transmitted disease lymphogranuloma venereum. L₃ causes pneumonia in mice. *C. trachomatis* is considered to be responsible for 20% of the pharyngitis symptoms in adults.

**Clinical aspects**

After an incubation period of approximately 7 days, four different clinical stages can be distinguished. These stages overlap. Reinfection can occur and makes the classification rather artificial.

**Stage 1**: there is bilateral redness of the conjunctivae. Photophobia, eyelid oedema and lacrimation follow. Small (2-3 mm) lymphoid follicles develop on the tarsal conjunctivae which increase in size over the course of one month. The inner side of especially the upper eyelid then becomes granular. This follicular-papular hypertrophy stage can last from several months to years.

**Stage 2**: After several months small blood vessels begin to grow into the uppermost part of the cornea. This process starts in the upper limbus of the cornea. The combination of blood vessels and infiltrate is known as a pannus. The mucus-producing cells in the conjunctiva are destroyed, leading to “dry eye” (sicca syndrome). Corneal ulcerations can occur. If left untreated the cornea becomes cloudy with functional blindness as the ultimate result. In rare cases the corneal neovascularisation regresses without treatment.

**Stage 3**: Linear scarring appears in the tarsal conjunctiva. Follicles are replaced by small white lines. The conjunctiva becomes smooth, white and avascular. The conjunctiva of the lower eyelid may take on a milky appearance. The craters of the ruptured follicles are lined with epithelium and form a
series of lacunae in the limbus, known as Herbert’s pits. The pannus regresses.

**Stage 4:** In this stage there is no longer any active infection. The scar tissue contracts and deforms the upper eyelid so that entropion follows. Due to the turning inward of the eyelid, the eyelashes scratch the cornea (trichiasis) and cause mechanical trauma. Bacterial superinfection can occur. The epithelium of the cornea becomes dull and thickened, which is made even worse by chronic exposure to dust and sand. This promotes further neovascularisation.

**Diagnosis**

In most endemic areas trachoma will be a clinical diagnosis. Chlamydia trachomatis can be cultured but the infrastructure for this is beyond the capabilities of most hospitals. PCR is more sensitive than culture. In the early stages small basophilic cytoplasmic inclusions can be seen with Giemsa staining in scrapings of the tarsal conjunctival epithelium. In clinical practice it is not necessary to provide formal proof of infection. Trachoma has to be distinguished from chronic allergic conjunctivitis. This is not always easy but eosinophilia and milky flat-topped papillae are present whereas basophilic inclusions are not found. Under field conditions the diagnosis of trachoma is likely to be correct if at least two of the following criteria are present:

1. Follicles on the upper palpebral conjunctiva in the mid-tarsal region
2. Linear scars of the tarsal conjunctiva (Arlt’s syndrome)
3. Active keratitis
4. Follicles in the limbus or their sequelae (Herbert’s pits)
5. Pannus in the upper third of the cornea.

**Treatment**

The treatment used to rely on the administration of tetracycline eye ointment or taking doxycycline 100 mg bid for 4 weeks (erythromycin for children). Currently the treatment of choice is a single administration of azithromycin (Zitromax®), which greatly simplifies treatment. At present WHO recommends annual mass azithromycin treatment for 3 years in communities in which the prevalence of “trachomatous inflammation – follicular” in children between 1 and 9 years of age is 10% or more. However the presence of clinical trachomatous follicular inflammation disappears more slowly than the implied by PCR results of conjunctival swabs. Further field-based study of estimating the prevalence of active infection is needed. Deformities of the eyelid, such as entropion or trichiasis have to be treated surgically. Reinfection can occur and further treatment forms part of a control programme.
Inclusion conjunctivitis (serotype D-K), a sexually transmitted disease has to be treated in the child and the mother as well as her sexual partners. It is important to make people aware of the fact that removing eyelashes which face inwards may bring some temporary relief, but that it can make the situation worse. The eyelashes grow back and the short stubby hairs scratch the cornea resulting in still more damage.

Trachoma is disappearing in many parts of the World even in the absence of specific control programs, probably due to the high background of antimicrobial drug use for other reasons.

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**Typhoid fever and other salmonellosis**

**Summary**

- Typhoid: important disease in terms of frequency and mortality.
- Over and under diagnosis are common
- *Salmonella typhi*: Human reservoir, causing systemic illness, hotspot Asia
- Non-typhoid *Salmonella*: zoonosis, causing enteritis (and invasive disease), hotspot Africa
- Typhoid fever: fever, abdominal pain, diarrhoea/constipation, dry cough, splenomegaly, relative bradycardia, rarely roseola typhosa
- Complications: ileal perforation, organ abscesses
- Clinical diagnosis: leukopenia, faeces/urine/blood/bone marrow cultures
- Widal test: serology has lack of specificity and sensitivity
- Treatment: (quinolones), ceftriaxone, azithromycin. Resistance is increasing worldwide.
- Importance of relapse, antibiotic resistance, chronic carriers, gallstones, schistosomiasis.

**General**

Typhoid fever is caused by infection with *Salmonella typhi*, a Gram-negative facultative intracellular bacterium. The genus is named after the American physician Daniel Salmon. Recently the bacterium has been named *Salmonella enterica subsp. enterica* serotype Typhi. However, the older name will be used in this text. It causes disease only in humans and has no animal reservoir, unlike non-Typhi *Salmonella* spp.

“Typhos” means smoke, obscurity, stupor in Greek and refers to the apathy, confusion, stupor and
neuropsychiatric symptoms which are often seen in severe infection. The word also reflects the earlier belief that illnesses were caused by all kinds of emanations (miasmas). The disease is sometimes difficult to differentiate from spotted fever, caused by Rickettsiae (in typhus the rash is more pronounced).

Paratyphoid fever is infection with the closely related bacteria *Salmonella paratyphi* A, B and C. Clinically the course of these is similar although rather milder. Gastro-enteritis caused by other animal *Salmonella* species should not be given the name paratyphoid. *Salmonella paratyphi* A and B have humans as their reservoir. The term “enteric fever” is a collective term that refers to both typhoid and paratyphoid fever.

Infections with non-Typhi *Salmonella enterica* can be invasive (i.e. positive blood cultures) and occur overall in about 5% of invasive cases. The invasiveness is age-dependent and varies according to serotype. For serotype Enteritidis and Typhimurium it increases by 10x above the age of 65 years.

**Bacterial structure**

The bacterium has flagella, structures which should not be confused which those of eukaryotic organisms. Anti-H antibodies bind to the flagella. Many *Salmonella* sp. can form two different H antigens. They sometimes undergo a phase change and possess either one or the other H antigen.

The bacterial wall structure is typical of Gram-negative bacteria. Around the cytoplasmic membrane lies a thin layer of peptidoglycans. This so-called murein layer consists of long chains of repetitive disaccharide links. Oligopeptide bridges connect the sugar chains. External to this second layer is a third; outer membrane. It consists of a phospholipid double layer in which complex lipopolysaccharides (LPS) are anchored. These fatty sugars have the following components, seen from the inside out: a fatty part (lipid A) anchored in the membrane, a core and an external sugar part consisting of repeating oligosaccharide chains. The latter form the so-called O antigens. The structure and sugar composition of the O antigens vary between different *Salmonella* species. However they all have the same basic structure, there are many serological cross-reactions. Lipid A is very toxic (endotoxin) and causes a broad spectrum of effects such as fever and shock during Gram-negative septicaemia. [Septic shock in infections with Gram-negative bacteria is mainly secondary to the effects of endotoxin. Shock in infections with Gram-positive bacteria is mainly due to the effects of secreted exotoxins]. Endotoxin acts on the proteins of the complement pathway and on various cytokine networks.

Specific antibodies are produced by the body: **anti-O and anti-H** (also called TO and TH). The
humoral antibodies result in little protection. Protection is based on cellular immunity. The O- and H-antigens are used in serological tests (Widal) [named after the French physician Ferdinand Widal, 1862-1929]. Since all Salmonella (not only Salmonella typhi) and all bacteria related to Salmonella possess similar antigens, there are many cross-reactions (the test is aspecific). The test also has low sensitivity. This means that the contribution of serology is limited in many clinical situations.

The Vi-antigen (virulence antigen) is a part of the capsule that surrounds the cell wall. It consists of a polymer of a single sugar. The Vi antigen physically covers the O antigen and thus protects it from anti-O antibodies. If the Vi-antigen is present, phagocytosis is more difficult and the bacterium will be more virulent. The infectious dose (ID50) for strains that possess this antigen is $10^7$, which is 10 to 100 times lower than the IG50 of strains without the Vi-antigen. The Vi-antigen also occurs in Salmonella paratyphi C and S. dublin (a subtype of S. enteritidis).

The bacterium produces and excretes a protein known as invasin, which allows non-phagocytic cells to take up the bacterium where it is able to live and replicate intracellularly.

**Epidemiology**

**Historical note on Typhoid fever**

It was quite a long time before typhoid fever was differentiated from other febrile disorders. Many scientists have contributed to our knowledge about typhoid fever. The French physician Pierre Charles Alexandre Louis first proposed the name “typhoid fever”. Between 1822 and 1827 he studied a total of 138 patients with typhoid fever, 50 of whom died. The post-mortem findings were compared to post-mortem results from 83 people who died from other causes. These ideas of meticulous documentation, the use of controls and numerical analysis of the data were an important milestone in medical history. Early ground-breaking work on the germ theory of disease and on the concept of water-borne transmission of illnesses water was done by Dr. William Budd (1808-1882). He investigated an outbreak of typhoid fever in the small Welsh border town of Cowbridge. In 1853, during the local race week, there were balls on two successive nights; eight of those who celebrated subsequently died of “typhoid fever.” The diagnosis of typhoid fever was confirmed at autopsy. Dr. Budd noticed that the local well was close to the septic pit of the inn, suggesting water contamination. He also noticed that a patient who was recovering from typhoid fever had left the inn two days before the parties took place. All the people who became ill had been given lemonade, prepared with water from the contaminated well. The water from the well was the only possible source of infection common to all those who died. He reinforced his theories
in 1866 when he and a colleague, Dr. Grace, traced a similar outbreak in several farm cottages. The fever was brought there by the father of one of the families. No one else was ill at the time he arrived and it was obvious that he had contracted the disease elsewhere, probably in nearby Bristol. Dr. Budd and his colleague noted that 4 weeks later several other cases of typhoid fever occurred in persons who lived in cottages which lay a quarter of a mile below the original outbreak. Those who lived in cottages at a higher level escaped entirely. They found that the drains from all the cottages were linked to the same stream and that the second outbreak had occurred downstream. They reasoned that the agent which provoked typhoid fever was carried there, contaminating the drinking water of the second group of cottages. Dr Budd claimed that typhoid fever was disseminated via the faecal-oral route, a new concept. Nevertheless, the community and the medical world were not yet ready for this theory. The earlier hypothesis that typhoid fever could be caused by “rotting material” (pythogenic fever). This was disproved in 1858-1859, when the great “Thames Stink” occurred in London. Due to certain hydrological and meteorological circumstances there was a huge wave of stench in London. The enormous amounts of rotting material in the river should have produced ideal conditions for typhoid fever, yet there were noticeably few cases during those years. The famous Canadian physician Sir William Osler (1849-1919) campaigned for a long time against the term “typhomalaria” which had been introduced by Dr Woodward to describe difficult febrile cases. In 1911, Elie Metchnikoff (1845-1916) fulfilled one of Koch’s postulates reproducing disease in chimpanzees after throat inoculation with *Salmonella typhi*.

**Military impact**

Throughout history epidemics of infectious illnesses have often played an important part in military conflicts. Famous examples are the American Civil War (1861-1865) with 75,361 cases of typhoid fever, of which 27,056 died. Note that these data are from the time before the bacterium had been isolated. In the Boer War in South Africa (1899-1902) 56,686 cases of typhoid fever were recorded, with 8,225 dead, compared to 7,582 who died from battle wounds. In the brief Spanish American War (1898) there were 20,738 cases out of a total of 107,973 soldiers, with 1,500 deaths. In those days, there was total disregard of the most elementary hygiene. This is in sharp contrast to the Russian-Japanese War of 1904-5, in which the Japanese boiled their water, tested their drinking water wells, covered latrines, disinfected excreta and sterilised cooking utensils, plates and mess tins. The low number of infected soldiers was probably due to these innovations. As well as typhoid fever, the role of epidemic typhus, epidemic borreliosis and bacillary dysentery in these conflicts should not be overlooked.
Transmission

Transmission is mainly via contaminated water and food. The bacteria survive for a varying number of weeks in water, ice, dust and can multiply in food. In many regions the infection is endemic. Sometimes there may be local epidemics. One classic mechanism is the contamination of a drinking water reservoir with the contents of a septic tank. In the past this was checked with a fluoresceine test. The bacteria only infect humans. There is no animal reservoir unlike the majority of the other Salmonella species. Recent convalescent patients form the most important reservoir. People can be healthy carriers and excrete Salmonella typhi for prolonged periods (concept published in 1903 by Robert Koch: the healthy chronic “Typhusbazillenträger”).

Typhoid Mary

A classic example of a healthy carrier is the case of “Typhoid Mary” the nickname of an Irish woman who became very famous at the beginning of the 20th century. In 1904 there was an epidemic of typhoid fever in a district of Long Island, New York. It was discovered that patients belonged to households where Mary Mallon had been cook. When she was tracked down by George Soper in 1907, she initially refused to cooperate. She was taken by the police, tested positive for S. typhi and was subsequently forced to stay at Riverside Hospital on North Brother Island. After three years she was released after pressure from the media. She then caused further cases including some at the Sloane Maternity Hospital where she worked in the kitchen under a false name. Overall it is certain that there were 53 cases, with 3 deaths, but possibly there were many more (possible role in the outbreak in Ithaca of 1903, with >300 cases).

Pathophysiology

After infection the bacterium penetrates the intestinal mucosa via M cells that overlie the ileal Peyer’s patches. M cells are phagocytic cells in the mucous membrane whose function is to sample microbes from the intestinal lumen and pass them on to the lymphoid tissue of the Peyer’s patch in order to activate the immune defences against intestinal microbes. Once inside the M cell the Salmonella replicate within the phagosome, subsequently killing the cell and spreading to adjacent cells. The bacteria are then taken up by mononuclear cells in the intestinal lymphoid tissue. There is intracellular multiplication in the mesenteric lymph nodes. From the lymphatic tract the bacteria pass into the blood (bacteraemia) and are disseminated through the whole body (spleen, liver, gall bladder, etc.). The intestine will be re-infected through the bile. The seeding of extra-intestinal organs can result in extra-intestinal complications virtually anywhere.
Clinical aspects

Overview of the symptoms during “classic” typhoid fever. Copyright ITM

Early clinical

Symptoms are quite variable. The **incubation period is usually 10 to 14 days**. This is considerably longer than the incubation time of 1-5 days for most other intestinal bacterial pathogens. One of the factors that determines the incubation period is the number of bacteria in the inoculum. There is always fever, which rises progressively. Initially there may be a brief episode of diarrhoea. Inflammation of the lungs leads to a dry cough. The combination of cough with fever sometimes leads to an assumption that the illness is a respiratory tract disorder. General malaise and headache are prominent. The illness may initially be confused with malaria. The patient is severely ill and sometimes apathetic or confused (typhoid = stuporous).

Half of the patients will subsequently develop abdominal pain. Diarrhoea -often described as pea
Bacteria | 217

soup- or constipation occur in roughly equal proportions (40 %). One third of patients vomit. The intestinal mucosa of the small intestine at the antimesenteric border becomes inflamed. The lymph follicles (Peyer’s patches) that are present in this location become infected and necrotic. Intestinal ulcers result which may subsequently perforate. If this does occur, the perforation is found in the final 60 cm of the ileum. Invasion of the liver and spleen leads to mild or moderate hyperplasia of the reticulo-endothelial system resulting in hepatosplenomegaly. Small red spots (2 to 5 mm) which recede when pressed can be observed on the trunk on white skin in a small number of patients. These “roseola typhosa” are quite difficult to see on a white skin and almost impossible to make out on a darker skin. The skin rash disappears after a few days. The heart rate is sometimes relatively slow for the fever (Faget’s sign, French physician Jean Faget 1818-1884). Tachycardia would be expected when the temperature is 39.5°C or 40°C. Relative bradycardia is not a constant finding however and is also non-specific. For example, it also occurs in yellow fever. If a liver biopsy is taken, very typical lobular aggregates of Kupffer’s cells are seen in the parenchyma (typhoid nodules). They simulate granulomas and illustrate the hyperplasia of the reticulo-endothelial system.

Complications

If untreated the fever remains high for two weeks, after which there is progressive improvement during the third week. If ileum perforation occurs, it is usually during this period. Generalised peritonitis results. There is then a sudden deterioration of the general condition: tachycardia, hypotension and pain in the right iliac fossa. A similar deterioration occurs in the case of gastrointestinal bleeding. Without antibiotics the mortality in typhoid fever is 10%, chiefly due to intestinal perforation, internal bleeding, sepsicaemia with toxaemia and the formation of abscesses in other organs. If there are no complications (deep-seated abscesses, cholecystitis, osteomyelitis, etc.), the fever disappears in the third week. Spontaneous abortion may be triggered by this severe illness. Hair loss may be extensive. Often the bacteria can still be detected using coproculture or urine culture, after the symptoms have disappeared. This is still possible one year after the illness in patients who become chronic carriers (1-6%, on average 3 % of patients). Carriers are more frequent in patients with gallstones or schistosomiasis. Prolonged salmonellosis in schistosome-infected patients is due to an association of Salmonella sp. with the schistosome worms themselves through pili which specifically recognize and bind glycolipids on the surface of the worms. The worms thus provide a multiplication focus for these bacteria in the portal mesenteric system, with a persisting blood stream infection following. Most carriers are asymptomatic.
Relapse

Relapse occurs in 2 to 10% of patients 5 days to 2 weeks after the fever has subsided. This usually has a milder course than the first episode. The relapse is caused by multiplication of reactivated persistent intracellular bacteria which were previously "dormant". It is not due to antibiotic resistance; so that treatment of a relapse is the same as that for the first attack. If there is a lack of clinical improvement in the first disease episode notwithstanding antibiotic treatment, the bacteria are likely resistant and the antibiotic must be changed.

Differential diagnosis:

Differentiation from other febrile disorders is initially difficult. The differential diagnosis should include: respiratory tract infection (clinical, chest X-ray), brucellosis (undulating fever pattern, vertebral involvement, blood cultures for which specific media need to be used), malaria (thick smear, thrombocytopenia), subacute bacterial endocarditis (heart auscultation, splinter haemorrhages, embolic problems, painful Osler’s nodes at the finger tips, Roth’s spots on the retina, blood cultures), kala azar (chronic splenomegaly, bone marrow amastigotes), deep pyogenic abscesses (elevated neutrophil count, ultrasound, aspiration of pus), liver amoebiasis (leukocytosis, clinical examination, ultrasound, serology, aspiration), typhus (often pronounced rash, sometimes chancre, meningeal signs, DIC [disseminated intravascular coagulation]). Differentiation from viral infections may be very difficult. Differentiating typhoidal ileal ulcers from those caused by tuberculosis or Crohn’s disease is usually easy.
Diagnosis

Blood cultures in typhoid fever have higher sensitivity than coprocultures in the early stages of the disease. Later, the inverse applies. Copyright ITM

Clinical

The diagnosis of typhoid fever is usually based on clinical criteria and in the majority of cases it will be made without formal proof. Perforation of the terminal ileum is quasi pathognomonic for typhoid fever, but the diagnosis should be made before this complication arises. In clinical practice there are
actually few disorders that cause perforations in the terminal ileum: typhoid fever, tuberculosis, trauma (e.g. ingested tooth pick) and Crohn’s disease. In many developing countries, two diseases often act as default diagnoses: malaria and typhoid fever. This illustrates the difficulties and uncertainties with which clinicians are confronted, together with the fact that both diseases are relatively frequent and are treatable (low threshold for diagnosis). Further too much importance is attached to a Widal test and the interpretation of a thick smear is often not reliable in a local laboratory (the problem is not the thick smear itself but the reading of it).

**Bacterial culture**

Positive cultures still form the gold standard for diagnosis. Cultures (bone marrow, blood, faeces, urine, duodenal aspirate or string test) will often be positive, but are often not feasible in practice. The chance of obtaining a positive culture is higher if repeated cultures are taken while culturing a sufficient volume of blood per culture. Blood cultures are positive in 40 to 80% of patients. In untreated patients there are ten times more bacteria per ml bone marrow than per ml blood.

**Serology**

Serological tests for antibodies to O and H antigens can be carried out (Widal or newer antibody-based rapid tests). A Widal test is only positive in 50 % at the beginning of hospitalisation, and may be positive due to salmonellosis suffered previously (e.g. due to *Salmonella* enteritidis) or due to an earlier vaccination. Routinely requesting this test under third world conditions makes no sense. The test can be used to detect a rising titre (seroconversion). Antibodies to the O antigen rise swiftly, and return to negative or to low titres in a couple of months (in particular type IgM antibodies). Anti-H antibodies rise more slowly but will stay positive for longer (in particular type IgG antibodies). If the presence of advanced typhoid fever is suspected on clinical grounds and if malaria is ruled out and a single Widal test is carried out (preferably using O antigen), then a high titre of these antibodies is a relatively strong argument that the patient does indeed have typhoid fever. Nothing can be decided from a negative result.

**Other arguments**

A complete blood count and differential often shows normal or reduced white blood cells. The eosinophils will be low or zero. In intestinal perforation there is leukocytosis, and in intestinal bleeding there is significant anaemia. A chest X-ray is often normal, in spite of the frequent presence of respiratory symptoms.
Treatment

Salmonella Typhi in many parts of the world have become resistant against chloramphenicol and other first line antibiotics (e.g. ampicillin, co-trimoxazole).

In addition, chloramphenicol has no effect on the relapse rate and is of no benefit to carriers. Ceftriaxone and quinolones (ofloxacin, ciprofloxacin) have subsequently become first line choice but are more expensive and there is quickly growing resistance for fluoroquinolones. The resistance of S. typhi to antibiotics varies from region to region but is increasing everywhere. Azithromycin or ceftriaxone are the drug of choice in areas with high levels of fluoroquinolone resistance such as Southeast-Asia. In many patients the time to defervescence may take from several days up to more than a week.

Adjunctive treatments include laparotomy in case of intestinal perforation and drainage of abscesses is recommended.

Prevention

Hygiene

General sanitary provisions such as clean drinking water, toilets and availability of soap to wash hands play a central role. If an epidemic occurs, in the first instance the source of infection should be sought. When treating patients with typhoid fever attention should be given to the disinfection of linen, disposal of faeces and hand washing. Treatment of chronic carriers, in particular those involved in the preparation of food is important (cf. Typhoid Mary), but opinions vary on this. Patients who are ill or convalescing are the chief source of bacteria in the community. A second problem is that in the tropics carriers cannot usually be traced due to the lack of infrastructure. Most patients stop excreting bacteria in the weeks following typhoid fever, and no new antibiotic treatment should be started in the first months after the acute illness, unless the patient is working in food preparation. Chronic carriers with gallstones often harbour bacteria in the biofilm on the surface of the stones. If treatment is needed, a cholecystectomy is suggested together with a quinolone for a longer period (not chloramphenicol). If there is urinary schistosomiasis, this should also be treated with praziquantel. The worms may harbour bacteria in their intestinal systems or in their tegument.

Vaccination

The old TABC vaccine had quite a number of side effects and no longer used nowadays. At present
there is an oral live vaccine (Vivotif®), which uses an attenuated strain of S. typhi. This vaccine contains no Vi-antigen. Vivotif® is administered as follows: 1 capsule taken on an empty stomach on days 1, 3 and 5. This provides protection in 70 % of individuals for 3 years. The vaccine containing Vi-antigen (Typhim Vi®) is injectable (1 injection) and provides the same degree of protection. The production of a Salmonella typhi Vi-conjugated vaccine (Vi-rEPA) is a new development. In this; the immunogenic polysaccharide of the bacteria is conjugated with non-toxic recombinant Pseudomonas aeruginosa exotoxin A. Trials have shown efficacy of 91% in children between 2 and 5 years and may confer longer immunity.

**Non-typhoid *Salmonella* blood stream infection**

Non-typhoid *Salmonella* bacteraemia is most likely to occur in immunocompromised hosts such as those who are at either extreme of the age spectrum or those who have diabetes, cancer, HIV positive, or who use immunosuppressive medications. When bacteraemia occurs, extra intestinal signs and symptoms may include osteomyelitis, abscess formation, and meningitis. Salmonellae may adhere to endothelial surfaces, resulting in cardiovascular infections, such as infectious endocarditis and endarteritis. Although atherosclerotic blood vessels are more susceptible to bacterial adhesion, infection of normal endothelial surfaces can also occur. The organisms may infect pre-existing aneurysms or atherosclerotic plaques, leading to arterial-wall necrosis and rapid aneurysm formation. The most frequently involved site is the infrarenal abdominal aorta.

Antibiotic resistance levels are even higher and mostly combined. Third generation cephalosporins and azithromycin have become drugs of choice.

**Cholera**

**Summary**

- Toxin from intraluminal intestinal bacteria, *Vibrio cholerae* O1 and O139
- Acute profuse to catastrophic watery diarrhoea with severe dehydration and ion loss
- Low or no fever and limited abdominal cramps
- Rehydration essential; preferably Ringer’s lactate
- Antibiotics are of secondary importance
General

Cholera is an acute infectious disease, characterised by profuse watery diarrhoea. It is caused by a Gram-negative bacterium: *Vibrio cholerae* O1 (the characters O1 indicate the serogroup). It is a very small, motile, curved bacterium (vibrio is the Greek word for comma). Various subtypes exist, with classification according to biological and biochemical behaviour (biotypes) and serological characteristics (serotypes). Until 1992 it was thought that bacteria causing cholera must belong to *V. cholerae*, serogroup O1 and that they must be toxicogenic (must possess and express the genes for toxins). It was known that non-O1 *Vibrio cholerae* could sometimes cause mild gastro-enteritis or even bloodstream infection in immune depressed patients, but not cholera. In October 1992 in Madras (India), a mutated pathogenic bacterium (a new serogroup) was discovered, which also causes cholera. The isolate was given the name *Vibrio cholerae* O139 (nicknamed Bengalen). After a short bloom, the traditional strains (O1) have become more common again. A few years later, *V. cholerae* O139 Calcutta was identified.

Antibiotic resistance genes in *V. cholerae* are often positioned on plasmids and can be transmitted to vibrios from non-pathogenic intestinal flora.

*V. cholerae* can only cause disease if there are pili [Lat.: “hairs”] present. Pili are shorter and thinner than flagella. The pili adhere to the intestinal mucosa.

Sometimes other *Vibrio* species are responsible for diarrhoea, e.g. *Vibrio cholerae* non-O1, *V. parahaemolyticus*, *V. hollisae*, *V. minicus* and *V. fluvialis*. Our knowledge of these latter bacteria is clearly insufficient. *Vibrio vulnificus* is an aggressive species present in seawater and filter-feeding organisms such as oysters. This bacterium may cause bloodstream infection and wound infections, certainly in patients with liver cirrhosis.

Biotypes

There are 2 biotypes: classic *Vibrio cholerae* and *V. cholerae* biotype El Tor. Biotype El Tor agglutinates chicken erythrocytes and causes lysis of sheep erythrocytes, unlike the classic biotype. The name El Tor originates from the Egyptian town and quarantine camp El Tor in the Southern Sinai dessert, where the bacterium was isolated for the first time in 1905 (during the 6th pandemic) from an asymptomatic Hajj pilgrim from Mecca. The importance of this germ was long disputed (until 1961). At present El Tor has replaced the classic variant in most places, except in the Ganges and Brahmaputra delta. El Tor may also survive longer in the environment, is less dependent on transmission via water and produces more asymptomatic infections (symptomatic/asymptomatic...
infections = 7/100).

Serotypes

*V. cholerae* O1 of both biotypes can be subdivided into **serotypes according to the structure of the O antigen**. If only O antigen A and C are present, the bacterium is known as serotype Inaba. If only A and B are present, the bacterium is known as serotype Ogawa. If A, B and C are present, the name Hikojima is given. Serotype shift seldom occurs (from Ogawa to Inaba and vice versa).

The difference between these serotypes is only of importance for epidemiological studies. For example: in 1991 all cases of cholera in South America were caused by toxin-producing *Vibrio cholerae*, serogroup O1, biotype El Tor, serotype Inaba. The cholera epidemic in the Rwandan refugees in DRC (July and August 1994) was caused by El Tor, serotype Ogawa. These bacteria were resistant to tetracyclines, cotrimoxazole, chloramphenicol and ampicillin. The outbreak in Haiti in October 2010, 9 months after an earthquake was due to *V. cholerae* O1, Biotype El Tor.

Flagella, pili and fimbriae

Most motile bacteria move about with structures called flagella (spirochaetes move with the help of axial filaments). Do not confuse active bacterial movement with random Brownian movement. Do not confuse a bacterial flagellum with the flagellum of a eukaryote such as Giardia (cf. also the remark concerning cilia in Balantidium coli). The flagella are too thin (0.2 µm) to be observed with a standard light microscope. The bacterial flagellum carries out a rotating movement. Some bacteria have several flagella. When the flagella rotate anti-clockwise, they form a coherent bundle, so that the bacterium moves in a straight line. On the other hand when the flagella turn clockwise, there is no longer any co-ordination and the bacteria move randomly. By timing the duration of clockwise and anti-clockwise spinning, this mechanism can be used in chemotaxis. The motor is in the membrane and the immediate driving power is not ATP, but a proton gradient. The bacterial flagella must not be confused with fimbriae, thread-like appendages which have no function in movement, but play a part in adherence to cells or tissues (important for virulence). Flagella rotate, fimbriae do not. Pili (singular pilum) are important in conjugation, the bacterial equivalent of sex. These hollow rigid tubes permit DNA transfer between bacteria. F-pili [Fertility] are important in the spread of resistance to antibiotics. Pili may also act as receptors for bacteriophages.
Epidemiology

Cholera has always been endemic in India and Bangladesh, in the huge delta formed by the confluence of the Ganges, Brahmaputra, Jamuna and Meghna rivers. Probably there was no cholera in Europe or America before the 19th century.

Between 1817 and 1923 there were various great pandemics, probably caused by the classic V. cholerae (there is no certainty as to the exact strain). The first pandemic which started in 1817 did not reach Western Europe. In 1829 the bacterium was introduced into the countries around the Persian Gulf via a British army unit stationed in India. From Iran the infection spread to Iraq, Syria, Georgia and Astrakhan (north of the Black Sea). It then travelled towards Odessa, Moscow, Vienna, Warsaw and Hamburg reaching England via the port of Sunderland. The first cases in London were seen in February 1832. The third pandemic merged with the second and was amplified by the miserable conditions during the Crimean war.

When each pandemic began and ended is rather unclear. There was cholera in Belgium in 1832, 1848, 1854, 1859, 1866 and 1892. In 1866, 1 in 100 Belgians died of cholera.

The pathogen was discovered in 1884 by Robert Koch during the fifth pandemic (first work in 1883 in Alexandria, Egypt, confirmation followed by research in India in 1884, with isolation of the bacterium in culture). In fact the bacterium had already been described in 1849 by Pouchet and in 1854 by Filippo Pacini, an Italian physician. However the latter’s work on this was not known outside Italy. The germ theory and in particular the work of Koch were attacked by Pettenkofer. Pettenkofer was a proponent of the “ground water theory” believing that the fermentation of organic matter in the subsoil (“miasma”) released cholera into the air (no transmission from person-to-person) which then infected the most susceptible e.g. those with poor diet, constitution, etc. Both Pettenkofer and his loyal student Emmerich drank a vial filled with cholera bacteria as proof against Kochs type transmission of V. cholerae. Amazingly, Pettenkofer did not then get cholera, but Emmerich suffered severe diarrhoea for 48 hours.

After the sixth pandemic there was a strange silence for about 40 years, for which no good explanation exists. The seventh pandemic was caused by El Tor. It started in 1961 in Celebes (Sulawesi), Indonesia, reached India in 1964 and Africa in 1970. In 2 years the infection passed through 29 African countries. In 1973 it arrived in the Gulf of Mexico. Early in 1991 the infection
spread rapidly in Peru. In 3 weeks there were 30,000 cases. The bacterium then spread further into South America, causing 360,000 cases within the year. In the summer of 1992 a second, less severe outbreak occurred. Nevertheless by August 1992 “only” 5,000 deaths had been reported (from an estimated total of 600,000 cases), thanks to the wide-spread use of rehydration therapies. The case-fatality ratio varied depending on the region. After 1993 the disease assumed an endemic character in several countries, sometimes with local outbreaks. At the end of 1993 the cumulative total amounted to 900,000 cases in three years (1991-1993), with a cumulative mortality of 8,000. According to one hypothesis cholera bacteria infected the marine plankton off the Peruvian coast via the ballast water from a Chinese freighter. The possible role of changes in the nutrient-rich von Humboldt current is still unclear.

Spread of the seventh cholera pandemic

About 80% of the cholera in 1997 occurred in Africa, chiefly in the horn of Africa (118,000 cases were reported officially). The increase in cholera in this region followed heavy rains and flooding (possibly associated with the El Niño weather phenomenon).
Since 1992 *V. cholerae O139* is recognised as a cause of a disease which is clinically identical to classic cholera, but which also occurs frequently in adults. Classic cholera in India, on the other hand, is common in children. There is no cross immunity with *V. cholerae O1*. Bacteria of the O139 serogroup have a polysaccharide capsule (unlike *V. cholerae O1*), which may explain the increased risk of bloodstream infection.

**Cholera O139**

After 1992 this new serogroup spread across Bangladesh, India, Pakistan and Southeast Asia. By the end of March 1993 more than 100,000 cases had been reported in Bangladesh. Further spread continued, but somehow diminished again, as the classic form and El Tor took over, reducing the incidence of the new serogroup. The reason is unknown. Therefore it is difficult to make then new Bengalen serogroup responsible for an 8th pandemic. It was observed in India that, after the first spread of *V. cholerae O139*, new variants (clones) of *V. cholerae O1* El Tor once more gained the upper hand.

Cholera also surfaces regularly in Madagascar. From the beginning of December 1999 until the end of February 2000 more than 12,400 cases were reported. The disease can thus certainly not be regarded as an entity which only existed “in the past”.

**Recent Epidemics**

At the end of 2008 a large cholera outbreak appeared in Zimbabwe. By February 2009, this led to more than 60,000 cases with a mortality of more than 5%, reflecting the general degradation of the nation’s basic infrastructure and the crumbling Zimbabwean health care system. By mid-April 2009 the official count was 96,591 cases with 4,201 deaths.

In 2010, more than 38,000 cases of cholera were identified in Nigeria.

In January 2010, a devastating earthquake hit Haiti, with its epicentre 25 km from Port-au-Prince, the capital. A couple of weeks before Nepalese United Nations peacekeepers arrived in Haiti, a cholera outbreak occurred in Kathmandu, the Nepalese capital. The forces were stationed in Mirebalais, 60 km north-east of Port-au-Prince. Late October 2010, patients with cholera were recognized in some Haitian rural areas. In less than 6 weeks, more than 10,000 cholera cases were identified. The disease quickly spread to the capital, where many people were still living in temporary shelters and tents, without access to safe drinking water or proper sanitary
facilities. By January 1, 2011, the Ministry reported 171,304, with a cumulative mortality of 3651. The hospital case fatality rate was too high, and a target of hospital CFR of < 1% should be achievable. A possible epidemiological connection with the Nepalese forces was suspected and created tension between the local population and the UN forces. The current Haitian strain of cholera was identified as a virulent hybrid of the El Tor O1 biotype and the classic type, serotype Ogawa.

Transmission

Cholera is spread by the faecal-oral route, via contaminated water and food. The infectious dose of bacteria required to cause clinical disease varies according to the mode of transmission and varies according to bacterial strain, with hyper-infective strains occurring immediately after gut passage. In people with normal gastric function and if ingested with water, the infectious dose is one thousand to one million vibrios. When ingested with food, it is lower about one hundred to ten thousand vibrios. The low pH of stomach acid kills most vibrios. When a person uses antacids, proton pump inhibitors or ranitidine, a lower infectious dose is required to trigger infection. The same applies to chronic atrophic gastritis and status post-gastrectomy. Asymptomatic infections are common, especially in case of El Tor. People excrete bacteria for about 10 days. This is sufficient time to ensure continued contamination of the environment. Chronic carriers are very rare, but occur, sometimes with vibrios lodging in in the biliary tract.

In third world countries many people have no chlorinated, filtered, treated, pure drinking water. The lack of good toilets and sewers leads to contamination of the surface or ground water. Too often untreated sewage water is still poured into surface water. Sometimes sewage pipes and drinking water pipes are laid in the same trench, which may result in contamination if there are leaks or greatly varying water pressures in the pipes. If drinking water is contaminated in this way, bacteriological checks of the drinking water when it leaves the pumping station will not show anything amiss. In houses, drinking water containers with a wide openings often become contaminated, because people are inclined to scoop up water in their (dirty) hands. Containers with a small spout, from which water must be poured are safer.

There is also direct transmission from person to person, but it is rare. The number of bacteria on dirty hands is usually lower than the minimum infectious dose necessary for direct transmission. Health workers who respect basic hygiene are at extremely low risk. Filter feeders such as mussels or oysters (especially in estuaria) concentrate the bacteria in their bodies. When the organisms adhere to food particles (e.g. the chitin of crustaceans) and in the case of hypochlorhydria, lack of
gastric acid due to gastric surgery, antacids, anti-ulcer drugs or atrophic gastritis, the number of organisms needed to trigger infection is much smaller. Food may be infected by dirty hands during or after preparation. The bacteria can survive and reproduce in food such as cereals, rice or lentils and crustaceans. This intermediate replication step is very important. If someone dies of cholera and a meal is made for the mourners at the funeral by the persons who have washed the corpse, the risk of further transmission is very real. The bacteria are very sensitive to drying out, sunlight and acid. Meals which contain acid e.g. tomatoes and/or lemon, are much less dangerous than neutral or alkaline meals. Vegetables and fruit on the market are often sprayed with water to make them appear fresher and more attractive. If this is done with contaminated water, transmission may occur.

**Historical note: John Snow and contaminated water**

In the first half of the nineteenth century a cholera epidemic occurred in London. In 1848, a cholera outbreak started which would kill more than 14,000 people in London. Another outbreak in 1853 killed more than 10,000 people. The physician John Snow, already well known in 1853 as anaesthetist to Queen Victoria. Dr Snow had also a special interest in cholera. In 1854 he examined the various families presenting cases and calculated that the mortality in the houses that were supplied with water by the Southwark and Vauxhall Water Company was 31/1000 houses. This was 8.5 times higher than in houses supplied by the rival Lambeth Company. Although neither of the two companies offered purified water, the first company took its water from the Thames near London Bridge, downstream from the city sewage outlets while the second company pumped its water upstream from the city at Thames Ditton. In 1849 there had been no difference in mortality between the families that received water from Lambeth or Southwark. Before 1851 the Lambeth Company drew its water from a highly contaminated stretch of the Thames near Hungerford Market. It was this spectacular change (1849 compared to 1854) which made Snow conclude that contaminated water had a causal connection with cholera. Although both companies delivered water in the same streets, the water used in any particular house could be identified by its salt content (London Bridge is closer to the sea and its water is saltier than that at Thames Ditton). Adding silver nitrate leads to precipitation of silver chloride, proportionate to the amount of salt in the water. This was the basis of a simple test that could be carried out in every house.

Similar findings were made in Hamburg in 1890. The incidence of cholera was 34/1000 in Hamburg, where the drinking water was drawn from the river Elbe, and 3.9/1000 for the surrounding areas where other sources were used. In Altona, to the west and downstream from
Hamburg, contaminated water was also taken from the Elbe, but there was less cholera. How could this be explained? If anything, more cholera would be expected in Altona. The difference was that in Altona the water was first filtered slowly through sand before being supplied for consumption. These observations led to attempts to provide cities with clean drinking water and to construct adequate sewers. Cholera was the first disease for which surveillance was set up and because of this the disease still has code number 001 in the international classification list of diseases.

Reservoir

Humans are the only vertebrate hosts. *Vibrio cholerae* can survive long-term and probably permanently in brackish water, especially if there is a neutral or slightly alkaline pH and the water contains minerals and organic material. The bacteria are concentrated in phytoplankton (certain algae) and zooplankton which live in this water. Among the latter, copepods, a group of crustaceans, are important.

Cholera is clearly seasonal. A chronic aquatic reservoir is likely and this might be independent of continuous human faecal pollution. *V. cholerae* excreted by humans can be cultured in the laboratory. These bacteria however may assume a living form which cannot be cultured in vitro and which do not multiply in the environment. However, those bacteria are not dead as they are known to multiply when instilled in a rabbit’s ileum. They are called ‘viable but not culturable’. It may revert to a replicating form in its natural environment when there are favourable environmental factors and this has important epidemiological implications. The living, but non-reproducing form of *V. cholerae* can probably cause disease. Traditional culture methods for tracing *V. cholerae* in water miss these “dormant” bacteria. Tests based on fluorescent antibodies may offer a practical solution, as they stain both dormant and active bacteria.

Physiopathology

The bacterium multiplies in the small intestine, where it adheres to the mucosal brush border. The bacterium is not invasive, in other words it does not penetrate the intestinal wall or pass into the blood. It excretes a very powerful toxin which causes active fluid secretion towards the lumen. This fluid is isotonic, ion-rich and protein free. There are no intestinal ulcerations and the faeces do not contain blood. There is little if any fever. There is no tenesmus. The faeces contain significant amounts of sodium, potassium and bicarbonate. Because of this the intestinal content is slightly alkaline (*V. cholerae* thrives best in a slightly alkaline environment and the bacteria are therefore producing the conditions which are optimal for their own survival). The loss of large amounts of
alkaline faeces results in metabolic acidosis. People with blood group O have an equal risk of infection but are at a significantly higher risk of clinically severe cholera if they become infected. The reason is unknown.

**Hypervirulent and hyperinfective strains play an important role in epidemics.** Passage of *Vibrio cholerae* through the gastrointestinal tract results in a short-lived, hyperinfectious state of the organism that decays in a matter of hours into a state of lower infectiousness. Such strains have a much lower ID<sub>50</sub> (the number of micro-organisms that will disease 50% of a population in normal conditions = measurement for virulence) than strains occurring in natural water reservoirs. The classic strain is associated with more severe illness. Faecal excretion of *V. cholerae* for up to two weeks has been documented and occasional asymptomatic carriers occur. Asymptomatic patients typically shed bacteria in their stools at about 1000 *V. cholerae* bacteria per gram of stool, which is a low level of shedding, compared with the 100 million bacteria per gram in case of ricewater stools.

**Toxins**

*Vibrio cholerae* produces several toxins: **cholera toxin (Ctx)**, the zona occludens toxin (Zot) and the accessory cholera enterotoxin (Ace). The role of the two latter toxins is not entirely clear. The Ctx enterotoxin of *V. cholerae* consists of 2 parts: **A and B, where A stands for active and B for binding.** They stimulate adenylate cyclase. Adenylate cyclase increases intracellular cyclic-AMP, which inhibits salt absorption by the microvilli and promotes active chloride excretion by the crypt cells. Water and potassium bicarbonate passively follow the chloride. In the end there is an overall water loss to the intestinal lumen. Fluid loss originates in the duodenum and upper jejunum, the ileum is less affected. The colon is insensitive to the toxin and cannot absorb the large amount of fluid quickly enough. Catastrophic diarrhoea follows.

**Cholera toxin**

Part A is a monomer, while part B consist of 5 identical subunits (a pentamer). The polypeptides of part B bind to a receptor (Gm1 ganglioside, a glycolipid) on the epithelium of the small intestine, after which part A can penetrate the cell. Part A binds covalently to an intracellular protein (Gs-protein; s for stimulatory) which irreversibly activates it, leading to the persistent stimulation of another intracellular enzyme, adenylate cyclase.

The toxic A-subunit also has other effects such as disturbing the expression of some genes, increasing inflammatory cytokines and inhibiting antigen presentation by macrophages. On the
other hand, the B-subunits of cholera toxin have anti-inflammatory properties. These are under intense study at present for possible therapeutic use in immune abnormalities. While cholera toxin adheres to the intestinal villus cells and disables the cellular saltwater pumps, the Zot toxin loosens the junctions that binds intestinal epithelial cells together. This contributes to the loss of water to the intestinal lumen.

The in-vivo detailed mechanism is probably more complicated. Cholera toxin also stimulates the nervous system in the intestinal wall, the myenteric plexus. This results in the release of 5-hydroxytryptamine (serotonin) from the enterochromaffin cells, leading to in additional fluid loss to the lumen. Granisetron, a 5-HT3 receptor blocker, partially reverses this effect. More research is needed to determine the role of this mechanism in the physiopathology.

Clinical aspects

The incubation period is brief: sometimes only hours, more commonly 1 to 5 days (average 2 days). It is one of the few infectious diseases where -in case of a very severe infection- you can be well in the morning and dead by sunset the same day. Asymptomatic infections are common (about 93%), but chronic carriers are very rare. Sometimes there is an initial transient fever (more seen in children). Massive watery diarrhoea starts suddenly. The faeces very rapidly look like water in which rice has been boiled: watery with flakes of mucus. The faeces have a fish-like smell. The volume of faeces may rise to 500 ml per hour. Vomiting is common, but abdominal cramps are unusual. The onset of thirst, oliguria or anuria and weakness is rapid. In a short time the patient develops severe dehydration and can die within 24 hours. In other cases the diarrhoea is less severe, especially with infections with El Tor. As the patient’s condition deteriorates, hoarseness of the voice and temporary deafness are often observed. Children with severe cholera may present with drowsiness or coma.
The signs of dehydration are thirst, dry mouth and lips (if the patient has not vomited recently), hollow eyes and sunken fontanel in children. The skin turgor diminishes. The skin becomes wrinkled (washerwoman’s hands). Often, the voice becomes weak and hoarse, the pulse quickens and is difficult to feel. The radial pulse might be impossible to detect. Blood pressure falls. There is little or no urine production (prerenal failure). Respiration becomes faster due to metabolic acidosis secondary to loss of bicarbonate in the faeces (bicarbonate is alkaline). This acidosis causes vomiting and muscle cramps. There is also significant potassium loss in the faeces. If rehydration is carried out using fluid without potassium, severe hypokalaemia may result. Nevertheless, quite often normokalaemia is found, together with an increased anion gap. The increase in anions (= negative ions) is multifactorial due to the hyperproteinaemia (hemoconcentration), hyperphosphataemia (internal shifts and renal failure) and lactate acidosis (shock). Ketones play little if any role. An
elevated hematocrit (hemoconcentration) can be found in nonanemic patients, as can neutrophil leukocytosis in severe cases.

The mortality from classic cholera may reach 50 %, but can be brought down to < 1 % with correct therapy. Mortality is chiefly due to dehydration with kidney failure, hypokalaemia, hypoglycaemia and aspiration pneumonia during vomiting.

**Diagnosis**

Cholera should be suspected in acute massive rice-water diarrhoea, certainly if there have been several cases in a short time (epidemic). The clinical picture of severe cholera is so spectacular that differential diagnosis does not present many difficulties. Milder cholera may be similar to other forms of gastro-enteritis (but not to dysentery). A child above the age of five years who develops acute dehydration, or dies as the result of acute diarrhoea, is always suggestive for cholera.

The vibrios are very small and can best be seen in a fresh faecal specimen with the help of dark field microscopy. There is characteristic motility (“star shooting”) which stops immediately after adding anti-O1 antiserum. This does not give any information on possible toxin production.

**Confirmation is best made via a bacteriological culture.**

**Culture of Vibrio cholerae**

Culturing should preferably be on a special medium in a bacteriology lab, e.g. TCBS-agar [=Thiosulphate-Citrate-Bile salts-Sucrose], polymyxin mannose tellurite agar (PMT) or another selective medium. TCBS agar is green before inoculation; sucrose-fermenting organisms such as V. cholerae turn it yellow. TCBS agar is important for rapid isolation and identification, but V. cholerae also grows on routine agar media. For routine media, large numbers of bacteria per gram of stool should be present to allow detection. Patients or carriers with low burden of bacteria will be missed with routine culture media. Overgrowth by normal faecal flora limits recovery of colonies. In order to identify the serogroup and the serotype one subsequently finds out to which antibodies (antiserum) the colonies obtained exhibit an agglutination reaction. It is also possible to find out whether the vibrios are toxicogenic (produce toxin), e.g. by a PCR variant called a loop-mediated isothermal amplification (LAMP) assay. Definitive identification is made in a reference laboratory.

Specimens may be transported in a transport medium, e.g. Cary-Blair. This is a kind of mild alkaline buffered gelatine in seawater with low redox potential in which the bacteria will survive for 4
weeks. If it is not available, a filter paper can be soaked with faeces and transported in an airtight bag to a well-equipped laboratory. A sample treated in this way remains usable for 1 week, but the recommendation is “the faster the analysis, the more reliable”. Blotting paper, soaked with liquid faeces and if possible placed in a 1% saline solution, can be kept for several weeks at 37°C (not in the freezer). This is useful if there are initial transport problems. Nevertheless it is better to have a fresh faecal specimen. For specimens from the environment or from food, in which the number of bacteria is much lower than in faeces, enrichment is necessary. The specimen can be incubated for 8 hours in alkaline peptone water, after which a TCBS agar is used.

About 10 days after infection with V. cholerae O1 the patient produces vibrocidal antibodies. They start diminishing after only one month and disappear within the year. Antibodies against cholera toxin are produced more slowly and remain for years. However these cross-react with enterotoxin produced by ETEC bacteria [enterotoxic Escherichia coli]. The immune response to V. cholerae O139 is not well understood. **The detection of antibodies is not important for the urgent care of the individual patient** but does permit retrospective diagnosis.

### Other Vibrios

Sometimes other *Vibrio* species are responsible for diarrhea, e.g. *Vibrio cholerae* non-O1, *V. parahaemolyticus*, *V. hollisae*, *V. minicus* and *V. fluvialis*. Our knowledge of these latter bacteria is clearly insufficient. *Vibrio vulnificus* is an aggressive species present in seawater and filter-feeding organisms such as oysters. This bacterium may cause bloodstream infection and wound infections, certainly in patients with liver cirrhosis or otherwise immune depressed.

### Treatment

**Rehydration** is essential and must be instituted as soon as possible. **Two phases** are distinguished. First it is important to replenish what has been lost in the previous hours or days. Then one must compensate the persistent fluid loss (e.g. the amount of fluid that is lost every hour). In mild cholera without vomiting oral rehydration may suffice. In severe forms IV fluids should be administered.

There are several possible compositions of rehydration fluids. **Solutions containing salt, sugar, potassium and bicarbonate are recommended.** Lactate is also good because it is converted in the body to bicarbonate. In cholera it is preferable to use Ringer’s lactate (= Hartmann’s solution). Normal physiological saline is second choice because it does not correct the acidosis nor does it contain potassium. Severe hypokalaemia may occur, with cardiac arrhythmias, kidney damage, paralytic ileus and significant muscle weakness with reduced or absent tendon reflexes. Dextrose (=
glucose) 5% without electrolytes is not advised as a rehydration fluid. A reminder: 1 gr KCl = 13 mEq KCl. So: Hartmann = Ringer’s lactate > Ringer > physiological saline >>> not glucose infusion if there is an alternative.

In severe cholera (fluid loss > 10% of weight) the missing fluids should be administered quickly, e.g. 6 litres over 4 hours for a patient weighing 60 kg. The first 3 litres may each be administered in 10 minute boluses (total therefore 30 minutes). After administration of the lost volume, losses are compensated with further IV and/or PO fluids (faeces volume + urine volume + 500 ml). Vomiting may make oral administration of fluids difficult. **Generally a total of 6 to 10 litres per patient is necessary.** When patients start to drink and stop vomiting, it is advised to leave IV lines in place for a while until you are sure rehydration will not pose any more problems.
Cholera epidemic in Congo. Refugees live in very poor conditions. Photo courtesy Els De Temmerman

Special cholera beds are useful: they have a central opening to allow the liquid faeces to pass through, and they can be collected in a bucket. This makes it possible to quickly determine the amount of fluid loss. During an epidemic people who can still hold themselves upright can sit on a bucket and try to drink as much ORS [oral rehydration solution] as possible. Children quickly develop convulsions and coma. It is important that hypoglycaemia should be considered. For an adult 50 ml of a 50% glucose solution is given IV, for a child 2-4 ml/kg 25% glucose or 10 ml/kg of a 10% glucose solution.

Antibiotics may be useful because they reduce the duration and thus the total volume of the diarrhoea and may therefore reduce the need for rehydration fluids. On the other hand, they are not
essential (given the non-invasive character of the infection) and resistance often occurs. At present a single dose of azithromycin 1 gram (child 20 mg/kg) or a single dose of doxycycline 300 mg (child 4 mg/kg) are possible treatments. Doxycycline is usually contraindicated in pregnant women and children under 8 years. However, the administration of a single dose should not provoke major adverse effects. Single-dose ciprofloxacin may also be effective. V. cholerae O139 is often resistant to cotrimoxazole (sulphamethoxazole-trimethoprim). There is insufficient data examining the effect of antibiotics on secondary transmission of cholera. However in published studies to date antibiotics have not been shown to decrease secondary transmission of cholera within households. Anti-peristaltic drugs such as loperamide may cause accumulation of fluid in the intestinal lumen with unfavourable consequences and should be avoided.

**Prevention**

In the industrialised world a patient with cholera will remain a sporadic case. In developing countries one case can lead to several secondary cases. Therefore ‘**enteric contact precautions**’ are essential in health care settings, focusing on very strict hand hygiene and thorough environmental cleaning and disinfection. The contamination of clothing and bedding is unavoidable. Boiling in water for five minutes is sufficient for disinfection. Mattresses and blankets can be dried in the sun. It is better to do this before washing them, to prevent infection of the washing area.

After surviving cholera a patient is probably immune for homologous biotypes for more than 3 years. There is some controversy: infection with the classic biotype seems to protect against recurrent infection by either biotype, but El Tor does not. No cross-immunity between V. cholerae O1 and V. cholerae O139 is seen, although they produce the same toxin. Immunity relies on antibodies in the intestinal lumen (the bacteria are not invasive). Systemic vibriocidal as well as anti-toxin antibodies develop during illness. Babies which are being breast-fed receive protective antibodies in their mother’s milk.

**Vaccination**

Former parenteral vaccination with dead V. cholerae bacteria (IM administration) did not lead to sufficient formation of protective antibodies in the intestinal lumen. Only about 50-65% of people living in endemic areas were protected for 3-6 months. The IM vaccine was associated with local reactions in 50% and systemic reactions (fever, malaise) in 10-30%. Advice to vaccinate with this type of vaccine was discontinued in 1972 by the WHO [World Health Organisation]. Parenteral vaccination, mass chemoprophylaxis and “cordon sanitaire” (= restrictions on travel and trade) are not effective in preventing or limiting outbreaks. A **newly developed oral cholera vaccine** is based on a killed
whole cell cholera vaccine combined with the recombinant B subunit of cholera toxin (Dukoral®). The vaccine contains 1 mg of recombinant B subunit, as well as $25 \times 10^9$ bacteria each of *V. cholerae* O1 classic Inaba, *V. cholerae* O1 classic Ogawa, *V. cholerae* O1 El Tor Inaba (heat-inactivated), *V. cholerae* O1 El Tor Inaba (formaline inactivated). Dukoral does not contain the A subunit of cholera toxin and therefore, no pathogenic toxin is present. Two to three doses need to be given. Two recent studies showed an effectiveness of 86% and 40% respectively; the latter study indicating a 63% protection against severely dehydrating cholera episodes. Lower levels of protection continue for 3 years. Protection wanes rapidly in young children. A herd immunity effect is expected in areas where vaccine coverage is more than 50%. Because the risk of cholera for most travellers is extremely low, vaccination should be considered only for those working in relief or refugee settings or for those who will be travelling in cholera-epidemic areas and who will be unable to obtain prompt medical care. WHO recommends that current available cholera vaccines be used as complements to traditional control and preventive measures in areas where the disease is endemic and should be considered in areas at risk for outbreaks.

**Mass (antibiotic) chemoprophylaxis is not effective** because (1) the infection spreads faster than the organisation of drug distribution, (2) the effect of a drug only lasts 2 days, after which re-infection may occur, (3) the whole population needs to be treated simultaneously and people should then be isolated and (4) it is difficult to convince asymptomatic people to take a drug. In addition, the selection of highly resistant strains has been observed in settings using mass-administration of antibiotics.

**Correct eating and drinking habits, safe stool habits and personal hygiene** are the most effective means for individuals to limit their risk of cholera. Improved sanitation is the pre-eminent method of eliminating cholera and many other faecal-orally transmitted infections. This is directly linked to the degree of poverty in a region. Boiling drinking water is often difficult since fuel may be scarce and expensive. Since a significant proportion of *Vibrio cholerae* can adhere to plankton, the drinking water can be filtered through a fine cloth, which removes both plankton and a lot of bacteria in a single operation. This is of course less effective than obtaining water from a clean pipe or pump but it is cheaper. Chlorination of drinking water may be important (piped water or via water trucks). This is difficult to accomplish in rural areas. Chlorination is much less effective if the water is turbid due to organic debris.

Eating raw fish, shellfish (e.g. oysters, mussels) and crustaceans (such as crabs, shrimps) should be avoided. Washing hands is important for transmission control within a household. Infected faeces should not be disposed of in a poorly functioning drain (hospital: e.g. in pit with unslaked lime = CaO). When large groups of people come together (funerals, festivals, etc.) there should be latrines with
facilities for washing hands and plenty of soap.

An attempt must be made to trace the source of small, local outbreaks (see Historical note on John Snow). Contaminated water is the chief suspect in a sudden local epidemic, while in isolated cases the cause should be sought in contaminated food. This is of course not an absolute rule. Food cooked by street vendors and in restaurants poses specific problems. Flies probably play an underestimated part in transmission, but their numbers also reflect the sanitary conditions in a region.

**The following points should be emphasised during information campaigns:**

1. Drink only clean water (boiled or chlorinated)
2. Cook food completely and eat it while it is hot
3. Avoid uncooked food, unless it can be peeled
4. Wash hands after a bowel movement
5. Wash hands before preparing food
6. Wash hands before eating
7. Correct use of a good latrine (also for children)
8. With correct treatment cholera is rarely fatal
9. If cholera is suspected medical help should be sought immediately
10. In diarrhoea, give plenty of fluids (e.g. ORS)
11. Cholera vaccines should be used as complements to traditional control and preventive measures in areas where the disease is endemic and should be considered in areas at risk for outbreaks

**In case of an epidemic, it is important to have a large stock of IV rehydration fluid available** as well as the means of **preparing large amounts of oral rehydration fluid**. Normally such buffer stocks should be stored at various strategic points. The stocks for cholera treatment should not be segregated in storage, but should be rotated during normal use to avoid stock expiring. As soon as an epidemic is suspected, use as much oral rehydration as possible first so that stocks of the IV solutions last as long as possible. Cholera beds should be made ready. In a normal epidemic an attack rate of 0.2% can be taken as a rule of thumb (i.e. 200 cases can be expected in a population of 100,000). This is useful for estimating the size of stocks that will be needed. Sometimes the attack ratios are higher (e.g. the Rwanda-Zaire border in 1994).

All this requires a solid epidemic preparedness.
Diarrhea in the tropics

Summary

- A common and major problem; a major cause of mortality in children
- Mortality due to dehydration and invasive bacteria
- Etiology: viruses, Shigella sp., Vibrio cholerae, Giardia lamblia, Entamoeba histolytica, ...
- Clinical: degree of dehydration, ± blood in the faeces, ± fever, ± acute/chronic
- Rehydration (PO or IV); nutrition, sometimes aetiological treatment necessary

General

Diarrhoea is very common in the tropics. It is often self-limiting, but its general significance cannot be overestimated. It is a major cause of malnutrition and is one of the main causes of death particularly in children.

What precisely is meant by diarrhoea varies between patients:

1. an increased number of defecations per day (e.g. more than 3)
2. a decreased consistency of the faeces or
3. an increased volume of stools (e.g. > 200 g/24h)

all are used to define the problem.

The WHO definition of diarrhoea is at least 3 evacuations every 24 hours of unformed faeces. Unformed means here that they take the shape of any container into which they are evacuated. WHO emphasises the importance of change in stool consistency rather than frequency, and the usefulness of parental insight in deciding whether children have diarrhoea or not.

Diarrhoea causes fluid loss resulting in dehydration. The patient also loses electrolytes, which can lead to ion imbalances, such as hypokalaemia. Acidosis develops due to the loss of bicarbonate in the stools, to reduced renal function (less acids are excreted) and to ketosis (breakdown of body fat due to reduced food intake). Often the patient has no appetite and the nutritional status which is sometimes already poor deteriorates further. Sometimes the mother thinks she is doing good by “letting the intestines rest” and temporarily not giving food. Moderate undernourishment can then develop into severe malnutrition (marasmus and kwashiorkor). The latter is often seen if a patient has
had a number of episodes of diarrhoea in quick succession.

**Dysentery** is a severe form of diarrhoea. Fever is common in bacillary dysentery, but rare in amoebic dysentery. Dysentery has three characteristics:

1. Abdominal pain
2. Tenesmus (pain due to cramps in the rectum) and false defecation need
3. Frequent evacuation of small quantities of faeces that are mixed with blood, mucus and/or pus

**Steatorrhoea** or fatty diarrhoea is characterised by large quantities of faeces with an increased fat content (the stools float on water). This occurs in certain malabsorption syndromes. The cause usually lies in disorders of the pancreas or small intestine.

**Aetiology**

Diarrhoea is usually caused by infections. Of the nearly 11 million deaths that occur annually among children under five years of age, diarrhoeal disease is the second leading cause (after respiratory tract infections). The most common cause of severe gastroenteritis worldwide is rotavirus which accounts for 29 to 45 percent of nearly 2 million deaths. Bacterial intestinal infections (especially dysenteria) also contribute to the high mortality.

The most common causes of diarrhoea include (non-exhaustive list):

1. **Preformed bacterial toxins**, with the bacterium itself being no longer active in the intestine. Examples include staphylococcal diarrhoea (*Staphylococcus aureus* toxin), the ingestion of *Clostridium perfringens* toxins after eating contaminated meat (pigbel) and *Bacillus cereus* toxins (contaminated rice, among other things). Incubation time very short (hours).
2. **Bacteria** which multiply in the intestines: *Salmonella, Shigella, Yersinia enterocolitica*, a whole zoo of related *Escherichia coli* strains, toxicogenic *Vibrio cholerae, Campylobacter jejuni*, toxicogenic *Clostridioides difficile*
3. **Protista**: *Giardia, Entamoeba histolytica, Balantidium coli, Dientamoeba fragilis*, microsporidia, various coccidia (*Isospora belli, Cryptosporidia, Cyclospora, Sarcocystis*). Sometimes malaria is accompanied by diarrhoea!
4. **Worms**: only in case of serious infections, e.g. *Schistosoma mansoni, Capillaria philippinensis, Strongyloides stercoralis, Trichinella spiralis*; rarely by other worms. *C. philippinensis* and *S. stercoralis* can remain several decades in the body and are able to multiply inside the human host, something that most other worms cannot achieve. They can be lethal. Since worm infections are so
common in the tropics, worm eggs are often found in the stools. However, there is not necessarily an etiological connection between the presence of helminth eggs and diarrhoea.

5. **Viruses**: Rotavirus, Astrovirus, HIV, Noroviruses (Noroviruses cause gastro-enteritis, with important vomiting accompanying the diarrhoea).

6. **Non-infectious causes** such as laxative abuse, animal or vegetable toxins, marine biotoxins, mycotoxins, irritable bowel syndrome and inflammatory bowel diseases (Crohn’s disease, ulcerative colitis) are much less common. Endocrine problems (hyperthyroidism) and related problems (vipoma, carcinoid, etc) exist but are present in a minority of persons who present with chronic diarrhoea.

It is not always important to discover the exact cause of an episode of diarrhoea: for example, it is important to distinguish between amoebic colitis and bacillary dysentery, but the difference between Rotavirus and Norwalk virus (= Norovirus) enteritis is at present not clinically relevant in the tropics.

Intestinal infections caused by protista occur everywhere but are more prevalent in tropical climates. The climate helps protista to survive in the outside world and poor hygiene promotes their transmission. Diarrhoea is often found together with a parasitic infection, but the causal connection must always be assessed critically. It is important to distinguish between infection and disease. Of the many protista that are found in faeces, only a few types are potentially pathogenic. Occasionally, *Plasmodium falciparum* and *Leishmania donovani* can cause digestive symptoms. The diarrhoea then displays no particular characteristics.

Patients with diarrhoea may be classified based on the disease duration, aspects of the stool and other clinical symptoms.

**Acute diarrhoea**

**Acute non-bloody diarrhoea with little or no fever**

If the diarrhoea is very watery and very abundant, the possibility of *cholera* must always be considered (see separate lecture notes on cholera).

Food poisoning by *bacterial toxins* (including staphylococci) may also causing this type of diarrhoea, resulting in explosive diarrhoea shortly after a meal. The bacteria reproduce in food and produce a thermostable toxin. The bacteria are usually killed when food is cooked or left over food is reheated. The toxin however is not destroyed by the heat and enters the intestine, where it causes massive diarrhoea, probably by neurotoxic action on the autonomous nervous system. **Antibiotics are**
therefore of no value here. Symptomatic treatment is indicated. Toxins produced by *Bacillus cereus* (often present in contaminated rice) can produce a similar picture or the “emetic syndrome”. Some milder infections, such as traveller’s diarrhoea, produce hardly any fever. In these cases bowel motion inhibitors (loperamide) can be given.

### Acute non-bloody diarrhoea with fever

In children any infection of any type can be associated with diarrhoea, e.g. otitis media, tonsillitis, pneumonia, urinary infection, etc. The main pathogens are viruses, some *Escherichia coli* (ETEC is the most common pathogen in traveller diarrhoea) and mild forms of bacillary dysentery (*Salmonella, Shigella, Campylobacter* and *Yersinia*). The possibility of malaria and typhoid fever must be considered.

Acute diarrhoea with fever but without bloody stools, **generally requires no antibiotics**. The emphasis is on administering fluids and electrolytes. In small children a bacterial infection of the intestine can rapidly give rise to bloodstream infection. Antibiotics may therefore be indicated in small children (<1 year), other vulnerable patients or in patients with persisting and/or deteriorating symptoms.

### Acute bloody diarrhoea with fever

This is the picture of a **bacillary dysentery**. Pathogens are *Shigella, Salmonella, Campylobacter* and some *Escherichia coli*.

Some bacteria are very aggressive, while others give rise to milder infections.

Complications can occur:

- toxic megacolon
- rectal prolapse
- bloodstream infection
- haemolytic-uraemic syndrome. TTP-HUS, often triggered by Shiga toxin produced by *Escherichia coli* O157:H7 or other verotoxin producing bacteria (VTEC). If HUS occurs, antibiotics are contraindicated because otherwise still more toxins are released from the bacteria that have been killed, which aggravates the clinical status.
- reactive arthritis
• Reiter’s syndrome [urethritis, arthritis, conjunctivitis, uveitis, hyperkeratosis of the palms of the hand (keratoderma blennorrhagicum) and painless ulcers in the mouth and on the glans (balanitis cicinata)].
• After using antibiotics an overgrowth of Clostridioides difficile can occur in the intestine. The toxins that are produced by this bacterium cause a severe inflammation of the colon (pseudomembranous colitis) which can develop into toxic megacolon.
• A very serious complication after Campylobacter enteritis is the Guillain-Barré syndrome, which is characterised by ascending paralysis caused by a demyelinating process of the spinal roots.

In case of bacillary dysentery, examination of the faeces under the microscope shows numerous white blood cells (pus) and red blood cells. Bacillary dysentery is associated with a marked disappearance of the normal bacterial intestinal flora. It is not possible to distinguish between the different bacteria by microscopy alone (culture is needed for this). Testing for different pathogenic Escherichia coli strains is difficult. E.g. testing for enterotoxigenic E. coli requires recovery of individual bacterial clones from an agar plate inoculated with a stool sample. This should be followed by molecular evaluation for detection of specific genes. This approach is not available in most laboratories, including most labs in the West.

As always, fluid and electrolytes form the basis for treatment. With bacillary dysentery, antibiotics are an important part of therapy. The resistance of the various bacteria varies. Multi-resistant bacteria are becoming more common, especially in South and Southeast Asia. Depending on the local conditions and resistance patterns, a quinolone (e.g. ciprofloxacin) or a neo-macrolide (e.g. azithromycin) should be used. The use of diarrhoea-inhibitors (loperamide) is not recommended.

**Acute bloody diarrhoea but little or no fever**

The main causes are amoebic dysentery and to a lesser extent mild bacillary dysentery. Examination under the microscope of fresh (still warm) faeces is important in order to identify motile trophozoites. The normal bacterial intestinal flora is maintained in amoebic dysentery. In case of severe amoebic colitis fever may be present. Amoebic dysentery is treated with medication against the trophozoites (tinidazole = Fasigyn®, metronidazole = Flagyl®) followed by medication against any remaining intestinal cysts (paromomycin = Gabbroral®, diloxanide furoate = Furamid®). Other less common causes of bloody diarrhoea without fever are acute schistosomiasis (eosinophilia, worm eggs), massive trichuriasis (microscopy), ulcerative colitis (rare in the tropics) and Balantidium coli (microscopy).

Ileocaecal intussusception can present with acute bloody diarrhoea followed by intestinal
obstruction. Sometimes **malignant tumours** can present with acute bloody diarrhoea.

**Food poisoning with Clostridium perfringens** causes necrotising enteritis. After the Second World War this became known as “darmbrand”. In the dialect of Papua New Guinea the disorder is known as “pigbel”. Pigbel has been recognised in Papua New Guinea since 1961. The disorder is seen mainly in undernourished and parasite-infested children after eating a rich meal with sweet potatoes and infected pigmeat (pig intestines are also eaten). Meals such as this are sometimes prepared on the occasion of a great feast at which the host expresses his social standing by slaughtering and serving a large number of pigs. The illness can therefore occur in epidemics.

### Clostridium perfringens

The anaerobic Gram-positive bacterium (Clostridium perfringens) is frequently present in the flora of the colon, so there must be other factors present to cause the onset of the disease. The bacterium, better known as the causative agent of gas gangrene, can produce various toxins. The bacterial strains which produce toxins can be classified into types A, B, C, D and E. All types produce alpha-toxin, which is a lecinthinase (phospholipase C). Clostridium perfringens type C, responsible for pigbel, produces alpha- and beta-toxins. The toxins in the intestine are usually destroyed by proteases. In case of undernourishment there is an important deficiency in proteases such as trypsin, and as a result the toxins can remain active. If there are trypsin inhibitors present as well, such as are found in sweet potatoes, the remaining small amount of trypsin is neutralised. Adult Ascaris worms produce trypsin inhibitors. If the intestine has reduced motility, the toxin remains in contact with the wall for a prolonged period of time and causes transmural necrosis. The lesions tend to be more prominent in the jejunum, although lesions of the ileum also occur. Besides supportive therapy, treatment is based on antibiotics (chloramphenicol, benzylpenicillin or other, broad-spectrum antibiotics), type C antiserum and mebendazole. Sometimes surgery has been performed. Vaccination against type C toxin is useful.

### Chronic diarrhoea

The great majority of diarrhoea episodes last less than one week, however when diarrhoea persists for 14 days or longer, it is called persistent diarrhoea. Some authors use the term “chronic” for diarrheal illnesses lasting 30 days or longer.
Chronic non-bloody diarrhoea without fever

In the tropics protista must be looked for in the first place: *Giardia*, *E. histolytica*, *Dientamoeba fragilis*, *Balantidium coli*, chronic intestinal capillaria, microsporidia, various coccidia (*Isospora belli*, *Cryptosporidia*, *Cyclospora*, *Sarcocystis*). There is a long list with other diseases causing chronic watery diarrhea: pellagra (niacin, vit B3 def), hyperthyroidism, irritable bowel syndrome, lactose intolerance, food allergies, coeliac disease, malnutrition, laxative abuse, neuro-endocrine tumours, intestinal lymphoma, collagenous colitis, AIDS, protein loosing enteropathy. *Campylobacter* infections and some strains of *E. coli* occasionally cause persistent diarrhoea.

Chronic non-bloody diarrhoea with fever

Chronic diarrhoea, emaciation and persistent fever are important criteria for the clinical diagnosis of AIDS. Other clinical signs should be searched for, such as oral candidiasis, Kaposi’s sarcoma lesions, chronic pruritus, severe or repetitive shingles. Serology can confirm the diagnosis. Intestinal parasites must be searched for.

Tuberculosis of the intestine is predominantly sited at the ileocecal transition. A mass can sometimes be felt there on palpation. There is sometimes ascites due to concomitant involvement of the peritoneum. Pulmonary lesions can be present, but these are certainly not a requirement for the diagnosis of intestinal TB. Intestinal tuberculosis occurs predominantly in immunocompromised individuals. It is difficult to differentiate intestinal tuberculosis from Crohn’s disease because of similar clinical, pathological, radiological, and endoscopic findings. Histological interpretation of biopsies is of limited diagnostic value in the differentiation of intestinal tuberculosis from Crohn’s disease, except when caseating granulomata are found. Mycobacterial culture (isolation of *Mycobacterium tuberculosis*) and PCR are helpful in making the distinction between intestinal tuberculosis and Crohn’s disease.

Chronic bloody diarrhoea without fever

One has to consider persistent amoebic dysentery, severe schistosomiasis (*S. mansoni*, *S. japonicum*), inflammatory intestinal diseases, intestinal tumour and repeated intestinal invagination. Be aware of diarrhoea due to other causes together with bleeding haemorrhoids.
Chronic fatty diarrhoea

Causes of steatorrhoea include abnormalities of the small intestine and insufficiency of the exocrine pancreas. Calcification of the pancreas in chronic pancreatitis can be seen in 50% of cases (X-ray of the abdomen). Concomitant diabetes mellitus should be searched for.

Non-infectious causes of intestinal abnormalities such as coeliac disease (hypersensitivity to gluten) and intestinal lymphoma are rare. Coeliac disease is associated with antibodies against gliadin (a component of gluten) and autoantibodies against tissue transglutaminase (and/or anti-endomysium antibodies). Tropical sprue is a disease of unknown origin, common in Asia but less so in Africa. The disease responds to treatment with tetracyclines and folic acid.

Some infections may result in malabsorption:

1. *Giardia lamblia*: microscopy of the faeces. These are often asymptomatic infections, so their importance should not be overestimated. Giardia can also give rise to secondary lactose-malabsorption: dairy products can no longer be tolerated.

2. *Capillaria philippinensis*: occurs mainly in the Far East but is rare. Infection is caused by eating raw fresh water fish. Like Strongyloides, this worm also leads to endogenous reinfection. It can therefore reproduce in the body unlike most other worms. The eggs and larvae can be found in the stools (repeated analyses are necessary). Treatment of intestinal capillariasis is with mebendazole. It is a potentially fatal infection.

3. *Strongyloides stercoralis*: serious infections cause diarrhoea, eosinophilia, pruritus and larva currens. The stools contain seldom eggs but larvae are present.

4. *Cryptosporidia* can cause malabsorption. The possibility of AIDS must be excluded in chronic cases. The parasite can be demonstrated using Ziehl stain.

5. *Cyclospora* can be compared with “large cryptosporidia” with variable acid-fastness on Ziehl stain. Treatment with cotrimoxazole is usually effective.

6. *Tropical Sprue*: the exact cause is not known, but an infectious origin seems probable. Macrocytic anaemia, glossitis, hypo-albuminemia and signs of vitamin-deficiencies (ADEK) are common. Treatment includes tetracyclines and folic acid.
Assessment of a patient with diarrhoea

The assessment of a patient with diarrhoea includes a thorough medical history on the disease duration, stool characteristics and other relevant clinical signs and symptoms as well as an estimation of the degree of dehydration. **Medical history** should focus on:

1. How long has the patient been suffering from diarrhoea? Is it acute (<14d) or chronic (>14d)?
2. Is there fever? Weight loss? Night sweats?
3. Is there blood or pus in the faeces, or is it watery diarrhoea?
4. Is the diarrhoea volume large (more likely small intestine) or small (more likely colon)?
5. Is there tenesmus? Suggests that the rectum has been affected by inflammation or ulceration.
   Diarrhoea or rectal discharge? (suggests proctitis)
7. Is the patient vomiting? Makes dehydration worse and makes therapy more difficult.
8. Are there a number of people in the area with the same symptoms? An epidemic?
9. Is the patient immunocompromised, or does he have major co-morbidity? Any (new) medication?

Acute diarrhoea is often caused by self-limiting infections (beware exceptions). Chronic diarrhoea is more often than not caused by non-infectious causes (beware exceptions, especially in immunocompromised patients). Two intestinal helminths which as a rule persist (probably for life) even in untreated immunocompetent persons are *Strongyloides stercoralis* and *Capillaria philippinensis*. Chronic diarrhoea can be further classified by volume, where small frequent stools are suggestive of a distal colonic disorder. Large volume watery stools are suggestive for conditions involving the small intestine (but beware of a secreting villous colonic adenoma). Steatorrhea or fat-malabsorption suggests problems located in pancreas, bile ducts and/or small bowel.

The presence of faecal leukocytes has a sensitivity of 70% for inflammatory diarrhoea. A test for faecal lactoferrin has a higher sensitivity but is rarely available. Continuation of diarrhoea during fasting is suggestive for a secretory process. Features that suggest an organic cause as opposed to a functional cause, include a duration less than 3 months, nocturnal diarrhoea, abrupt onset, weight loss (>5kg for an adult), stool weight more than 400 g/24h.

**Clinical assessment (degree of dehydration)**

The **assessment of dehydration is most important**. Dehydration is due to an insufficient intake of liquids (drinking, IV fluid) and/or excessive loss of fluid (vomiting, diarrhoea, polyuria, sweating). If loss of gastro-intestinal fluid is the cause, the patient will urinate less (oliguria) in order to minimise
If a child has lost < 5% of its body weight, the general condition is still quite good. The child is alert and thirsty. The mucous membranes (eyes, tongue, mouth) are moist and the turgor of the skin (elasticity) is maintained. Breathing is normal. Urine production is normal and if the child cries there are tears. The fluid deficit is < 50 ml/kg of body weight.

If 5-10% of body weight is lost the eyes are sunken, the fontanelle is hollow, the skin is no longer elastic, the lips and mouth are dry and sometimes cracked. The child is miserable, restless and cries. There are no tears. Breathing becomes more rapid (acidosis). This must be distinguished from an accompanying pulmonary infection. Urine production decreases. The fluid deficit is 50-100 ml/kg. With a fluid loss of >10% the child is quiet and cold. The pulse is rapid and difficult to feel (circulatory collapse), especially the radial pulse. Skin folds do not disappear, the mucous membranes are very dry, the abdomen is hollow, the eyes are deeply set and the fontanelle is deeply sunken. Usually there is no more urine. The fluid deficit is >100 ml/kg.

A rapid clinical dehydration evaluation can make use of the following items: general appearance, skin, eyes, tongue and tears. A more detailed evaluation can determine the following items:

**Table: Evaluation dehydration for children up to 36 months**

<table>
<thead>
<tr>
<th>Appearance:</th>
<th>Normal</th>
<th>Thirsty-restless-irritable</th>
<th>Drowsy-limp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary refill</td>
<td>&lt;1.5”</td>
<td>1.5-3”</td>
<td>&gt;3”</td>
</tr>
<tr>
<td>Skin turgor</td>
<td>instant recoil</td>
<td>&lt;2 seconds</td>
<td>&gt; 2 seconds</td>
</tr>
<tr>
<td>Fontanelle</td>
<td>Normal</td>
<td>Slightly sunken</td>
<td>Very sunken</td>
</tr>
<tr>
<td>Eyes</td>
<td>Normal</td>
<td>Slightly sunken</td>
<td>Very sunken</td>
</tr>
<tr>
<td>Tongue</td>
<td>Moist</td>
<td>Sticky</td>
<td>Dry</td>
</tr>
<tr>
<td>Tears</td>
<td>Present</td>
<td>Decreased</td>
<td>Dry</td>
</tr>
<tr>
<td>Breathing (&lt;1y)</td>
<td>&lt;40/’</td>
<td>40-50/’</td>
<td>&gt; 50/’</td>
</tr>
<tr>
<td>Breathing (1-3y)</td>
<td>&lt;30/’</td>
<td>30-40/’</td>
<td>&gt; 40/’</td>
</tr>
<tr>
<td>Heart rate (&lt;6m)</td>
<td>&lt;175/’</td>
<td>175-185/’</td>
<td>&gt; 185/’</td>
</tr>
</tbody>
</table>
### Treatment

#### General

Two things must always be considered: (1) the degree of dehydration/rehydration needs, (2) is drug treatment necessary? The most important thing with acute diarrhoea is to deal with dehydration and in the second place to correct protein and calorie deficiency. Etiological treatment will only be possible in a minority of cases, but should not be disregarded.

Children are very sensitive to dehydration. Fluid loss can occur very quickly with vomiting and diarrhoea: 500 ml of fluid in a child weighing 5 kg means a loss of 10% of body weight and implies a high risk of death.

**IV rehydration** is not always possible nor even desirable. An important development has been the discovery that many cases of dehydration of whatever origin can be counteracted by oral rehydration. This is possible because despite the diarrhoea, the mechanisms for absorbing water, sodium and glucose in the intestine are maintained. The minimum ingredients for this oral rehydration solution (ORS) are clean water, glucose and salt. While this can indeed bring about rehydration or prevent dehydration, a disadvantage is that the diarrhoea itself continues. The volume of stools is not reduced. Alternatives to glucose are ordinary sugar (sucrose; this is a glucose-fructose disaccharide) or rice powder. Rice powder is better because it reduces the volume of stools. In ideal circumstances potassium (against hypokalaemia) and bicarbonate or sodium citrate (against acidosis) can be added. Citrate is easier to store than bicarbonate. In the future there may perhaps be better formulae which also contain neutral amino-acids (glycine and alanine) and perhaps dipeptides.

There are several formulae for ORS. The WHO has developed a standard formula in which each litre of water should contain:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>1.5 gram</td>
</tr>
<tr>
<td>Trisodium citrate dihydrate</td>
<td>2.9 gram</td>
</tr>
</tbody>
</table>
Treatment, in practice

- Always weigh the child and assess its general condition.
- Assess whether the weight loss is <5%, 5-10% or >10%.
- Is it dysentery or not? If yes, is it amoebic or bacillary?

**With mild to moderate dehydration use ORS.** The volume that should be given is 1-2 times the fluid deficit. ORS is best given by the mother and should be given over a 4 to 6 hour period. It is best if it is given with a small cup and spoon. With very small children a syringe can be used to drip the fluid into the mouth. If the child vomits a few times the treatment should be continued nevertheless. Administration using a nasogastric drip infusion is rarely necessary. The success of the treatment should be monitored by assessing the general condition of the child and its weight.

**With severe dehydration (>10%) or if the treatment with ORS is not successful, IV rehydration should be used.** If it is not possible to inject into a vein and a venous cut-down is not feasible and the situation is desperate, the intraosseous route can be used: the fluid enters the bone marrow of the tibia and is taken up in this way. The infusion can be rapid at first (70 to 100 ml/kg over 3 hours). If the pulse can be felt clearly again and the child has generally improved, the treatment can then be switched to oral therapy. Potassium chloride should be added in severe diarrhoea.

Newborn children with a low birth weight are very sensitive to hypernatremia. Rehydration is achieved best with 2/3 ORS and 1/3 extra salt-free water.

Food must continue to be given while the patient has diarrhoea. It used to be thought that a period of fasting (24 to 48 hours) was good for the child, but this is counterproductive. Breastfeeding should not be stopped. A balanced diet, low in residue and semi-solid is indicated. During episodes of diarrhoea, patients are catabolic (they break down their own muscle proteins for energy).

**Medication**

1. Antibiotics for bacillary dysentery.
2. Antiparasitic agents for amoebiasis, giardiasis, malaria, isosporiasis, Strongyloides, capillariasis, etc.
3. Zinc, folic acid and vitamin A supplements, especially in malnourished children.
4. Antimotility products loperamide (Imodium) or opiates: codeine, paregoric (= opium tincture) or laudanum reduce intestinal cramps and the frequency of bowel movements. They are only indicated for uncomplicated diarrhoea. They do not reduce fluid loss. Anti-diarrhoeal drugs must be avoided in children because they can aggravate dysentery and can easily be given to children in too high a dose resulting in paralytic ileus and sedation interfering with oral rehydration.
5. Sometimes the main complaints is nausea. Domperidone can be used, though its use should be restricted to severe cases, especially when combined with other QTc-proloning drugs as fluoroquinolones (ciprofloxacin, levofloxacain, moxifloxaclin) or( neo-)macrolides (clarithromycin, azithromycin).
6. Lactobacillus and saccharomyces boulardii concentrates are probably of little benefit but more research is needed.

**Prevention**

Most diarrhoea is transmitted by the faecal-oral route. The prevention of these infections will therefore depend on improved general hygiene, which is determined by the general level of poverty (standard of living).

Rotavirus disease kills approximately half a million children annually in developing countries and accounts for one third of hospitalizations for diarrhoea worldwide. In 1999, the first licensed rotavirus vaccine (RotaShield) was withdrawn from the U.S. market less than a year after its introduction because it was associated with an uncommon but potentially life-threatening adverse event, intussusception, at an estimated rate of 1 incident per 10,000 vaccine recipients. The manufacture of the first licensed rotavirus vaccine was halted. In 2005, results of large clinical trials of two new vaccines, Rotateq from Merck and Rotarix from GlaxoSmithKline, were published. These are both live oral vaccines intended to be given to infants at the same time as their immunizations for diphtheria, pertussis, and tetanus, but they differ in their approaches, strains, and formulations. Rotarix is given in 2 doses with minimum 4 weeks interval. Rotateq is given in 3 doses with minimum 4 weeks interval. Both vaccines demonstrated an impressive efficacy profile and a reassuring safety profile, particularly with respect to intussusception.

A few general tips and precautionary measures for avoiding diarrhoea are recommended:

1. Food should be completely cooked/boiled.
2. Drinking water should be protected. This can be achieved in a village context (sand filters,
Bacteria | 255

protection of water-wells, etc). Water can be boiled and filtered, but boiling requires a lot of fuel, which is usually expensive.

3. Wash hands with soap.

4. Sanitary provisions: toilet and drinking water should be kept separate. Inexpensive, simple, build-it-yourself, ventilated, odour-free, fly-free latrines that do not require any water can be made (the Blair latrine for example).

Diarrhoea: prevention for travellers

Food: avoid raw vegetables, fruit you cannot peel yourself, unpasteurised dairy products, fish, shellfish and meat that is raw or not cooked through. (Cook it, boil it, peel it or leave it). Avoid food from street stalls. Food should be protected against flies.

Drink: drink tea, coffee or bottled water, preferably sparkling (less risk of having been tampered with). Beer can quench the thirst, but large quantities of alcoholic drinks are not recommended. Avoid bottles sealed with reused crown caps. Ice cubes are not to be trusted. Drinking water can be filtered. This can be done in a number of ways (large porcelain filters such as Berkefeld, active charcoal filters, portable Katadyne filters). Afterwards the water can be boiled or purified chemically with silver salts such as Micropur®, Drinkwell® (not active against viruses), Chloramine (250 mg per 10-50 litres) or sodium hypochlorite (Javel, Drinkwell chlorine®, Hadex®). An unpleasant taste of chlorine can be removed by adding the non-toxic sodium thiosulphate (Drinkwell-antichlorine® drops) work in for an hour. Lugol or 2% tincture of iodine (eight drops per litre) can also be used and is more active against amoebic cysts. Long-term use (more than 3 months) is not recommended. Thyroid disorders and pregnancy are contra-indications.

Chemoprophylaxis: This is normally not advised routinely, but does provide partial protection (e.g. ofloxacin). Only to be considered for short journeys where absolutely nothing should go wrong.

LAST UPDATED BY ADMIN ON AUGUST 16TH, 2023

Tropical sprue

General

Tropical Sprue is largely limited to within about 30 degrees north and south of the equator. It was responsible for one-sixth of all casualties sustained by the Allied forces in India and Southeast Asia
during World War II. Tropical sprue is an acquired disease of unknown origin. An infectious origin appears probable and the term “post-infectious malabsorption” is also used. Possibly there is an initial insult at the level of the jejunal-ileal enterocytes, followed by bacterial overgrowth with enterotoxic strains. The disease is characterised by abnormalities of the mucosa in the small intestine with chronic malabsorption, multiple nutritional deficiencies and anaemia. The malabsorption is generalised and affects absorption of proteins, fat, carbohydrates, minerals and vitamins (typical is iron and folate deficiency). Good response to treatment with doxycycline and iron-folate supplements is seen.

Tropical sprue occurs chiefly in the Caribbean, India, Nepal and Southeast Asia, in both the indigenous populations and immigrants. Cases have been reported from Mauritius, Fiji, southern Italy, Guyana and Central America. In Africa the disease is apparently very rare, although cases have been reported from Zimbabwe.
Clinical aspects

Tropical sprue can have an insidious onset or can start acutely. The symptoms are those of chronic malabsorption. Generally it presents as a clinical triad of painful tongue, weight loss and persistent abdominal discomfort with diarrhoea. Patients are noticeably tired, both physically and mentally. Amenorrhoea is very common. There is loss of weight with muscle atrophy. Hypoalbuminaemia leads to oedema. Due to malabsorption of carbohydrates there is increased gas production in the intestines, with borborygma, a bloated feeling in the abdomen and intestinal cramps. The D-xylose absorption test is abnormal in more than 90% of cases. Fat malabsorption leads to steatorrhoea with more than 10 g of fat in the faeces. This occurs in 95% of patients. The stools are pale, very odorous and quite voluminous, up to 5 times the normal amount. Dehydration, hyponatraemia and hypokalaemia are very common. Calcium deficiency may lead to tetany with positive Trousseau’s sign. Hypokalaemia leads to reduced tendon reflexes and U-waves on the electrocardiogram. There is usually a deficiency of vitamin B12, folic acid and sometimes also iron. Anaemia occurs and is typically macrocytic with megaloblastic bone marrow. In long-term cobalamin deficiency there may be peripheral neuritis and involvement of the spinal cord, chiefly of the dorsal columns (proprioception). The tongue is red and painful. As well as glossitis there may be stomatitis with superficial erosions. Deficiencies in fat-soluble vitamins (A, D, E, K) lead to prolongation of the coagulation time and osteomalacia. Vitamin A deficiency is characterised by a dry, rough skin with follicular hyperplasia and Bitot’s spots on the conjunctivae. In severe deficiency night-blindness and xerophthalmia may occur.

Differential diagnosis:

Tropical sprue is a pan-enteric inflammatory process often mistaken for gluten-sensitive enteropathy. The differential diagnosis is that of chronic malabsorption. It includes persistent giardiasis, isosporiasis, strongyloidosis, intestinal capillariasis, gluten enteropathy (coeliac disease), chronic pancreatitis, intestinal tuberculosis, intestinal amyloidosis, Whipple’s disease, the blind-loop syndrome, bacterial overgrowth, diverticula and jejunocolic fistulae. Crohn’s disease is rare in developing regions.

Diagnosis

Tropical sprue should be suspected in anyone with megaloblastic anaemia and malabsorption who has lived in an endemic region or has visited these regions. Biopsy of the jejunum shows typical abnormalities. Intestinal villi become shorter and broader (blunting without flattening as in gluten enteropathy). In the intestinal wall there is an inflammatory infiltrate, chiefly consisting of lymphocytes, plasma cells and a few eosinophils. The enterocytes exhibit large vacuoles. Radiography
of the small intestine shows non-specific changes. There is flocculation of the contrast material and segmentation of the barium column, distension of the lumen and thickening of the mucosa. The mucosal folds in the small intestine are irregular and thickened, which gives the impression of a stack of coins. In advanced cases, no mucosal folds at all can be seen. A flat mucosa is very unusual and should lead to suspicion of a different disease (e.g. gluten enteropathy).

**Treatment**

Treatment is based on tetracyclines 250 mg QDS or doxycycline 100 mg daily for 3 to 6 months. Folic acid supplements (5 to 10 mg daily) and multivitamins and if necessary iron should be added to the treatment. Response to treatment is generally swift with an initial improvement within three days. Further recovery takes place in the course of the following three months.

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