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Malaria

Summary

- Malaria is very common; a very important cause of mortality and morbidity in the tropics
- Five parasites: Plasmodium falciparum, *P. vivax*, *P. ovale*, *P. malariae*, and since 2007; *P. knowlesi*, (in Southeast Asia)
- Transmission via female Anopheles mosquitoes which bite at night
- Symptoms: fever and body-ache; sometimes atypical or “chronic” (anaemia, splenomegaly)
- Risk of complicated presentations, mainly with *P. falciparum* (severe anaemia, kidney failure, cerebral malaria)
- Infections often asymptomatic in semi-immune people (generally low parasitaemia)
- Clinical diagnosis not reliable.
- Often clinical over-diagnosis of malaria and under-diagnosis of other disorders in endemic areas
- Diagnosis via thick smear, thin smear, rapid antigen-detection, DNA-based methods
- Treatment of *P. malariae*: chloroquine
- Treatment of *P. vivax* and *P. ovale*: chloroquine or if possible with primaquine (hypnozoites, G6PD).
- Resistance of *P. vivax* to chloroquine is rising in several areas
- Increasing multidrug resistance of *P. falciparum*, including resistance to artemisinin derivatives
- Combination treatment of *P. falciparum* infection is strongly advised:
  - a) ACT: artemisinin combination treatment (e.g. artemether + lumefantrine; = Co-Artem, Riamet).
  - b) Quinine + (doxycycline or clindamycin)
  - c) Atovaquone + proguanil (Malarone)
- Individual prevention via pyrethroid-impregnated bed net ± chemoprophylaxis; stand-by emergency treatment (self-medication) for certain travelers?
Malaria in humans

Malaria is the common name for diseases caused by infection with single-celled parasites of the genus Plasmodium. Among the parasites of the genus Plasmodium five species have been identified which regularly cause disease in humans:

- Plasmodium falciparum
- Plasmodium vivax
- Plasmodium ovale
- Plasmodium malariae
- Plasmodium knowlesi

However, in 2017 several malaria outbreaks in Brazil were caused by *P. simium*, a malaria species closely related to *P. vivax* that was previously considered to be a monkey-specific malaria parasite.

Human malaria parasites have a restricted host-specificity. They don’t develop disease in rabbits, rats or mice but need to be maintained either in human volunteers or in primates. A common used less-than-optimal substitute is to perform experiments on primate, rodent or avian malaria parasites in their natural host. Most animal models are inadequate and, while they can help the researcher in answering specific questions, any extrapolation to human disease has to be considered with extreme caution. For example dexamethasone was considered to be useful in severe malaria caused by *P. knowlesi* in rhesus monkeys, but was found to be harmful in humans.

**Historical note**

**Discovery of the parasite**

Malaria has been with humanity since millennia. The most famous historical case of falciparum malaria is probably King Tutankhamen, the boy pharaoh from Old Egypt, in whose 3,000-year-old mummy the parasite was demonstrated. Although usually associated with the tropics malaria was endemic in North America and large parts of Europe until the middle of
the 20\textsuperscript{th} century. Malaria transmission occurred in Belgium, the Netherlands, Sweden, Finland and the United Kingdom. It was a significant impediment for the European nations during the colonial period. In Northern Europe, only \textit{P. vivax} and \textit{P. malariae} occurred. In Southern Europe, malaria was due to infection with \textit{P. falciparum}, \textit{P. vivax} and \textit{P. malariae}.

In 1880 the French army doctor Charles Louis Alphonse Laveran discovered malaria parasites in fresh blood from malaria patients in the coastal town of Bone (Annaba), Algeria.

**Transmission**

The transmission of malaria had for long been a mystery. One of the researchers was the Briton Sir Ronald Ross. He left for India with a personal mission to prove transmission via insects. In 1897, after three years of hard work, he demonstrated parasites in mosquitoes which had bitten patients. Later he also demonstrated transmission of avian malaria via mosquitoes. He was able to describe the complete development of the parasite in the mosquito and also demonstrated that transmission took place via the bite of the mosquito (and not via the presence of dead mosquitoes in drinking water, as his mentor Patrick Manson had initially thought).

**Life Cycle**

After the cause and transmission of malaria became known, it was logical to assume that the parasites inoculated via a mosquito bite would directly penetrate red blood cells. This wrong idea was proposed in 1903 by Fritz Schaudinn, a distinguished German microscopist. It was based on faulty observation and due to his authority, it entered some textbooks. It was known that when blood from a patient with active malaria was inoculated into a healthy volunteer, the volunteer would develop malaria and would become infectious nearly instantaneously. However, when a volunteer was inoculated via a mosquito bite, the blood was not infective for 6 days (in case of \textit{P. falciparum}) to 9 days (\textit{P. vivax}).

Why? This was a vexing problem which took decades to answer. It was by very careful animal experiments with \textit{P. cynomolgi}, a primate malaria species, that the puzzle was solved. Shortt and Garnham collected a large number of infected mosquitoes, mashed them to pulp and injected the lot (including sporozoites) into monkeys. After waiting a period, they killed the animals and searched the various organs and tissues. The parasites (with a different shape) were found in the liver. They had to support their hypothesis of the existence of a pre-erythrocyte stage with a species of human malaria. They used \textit{P. vivax}
and a human volunteer. This man was inoculated IV with sporozoites isolated from 200 mosquito salivary glands. A week later, the volunteer was operated on and a piece of liver tissue was obtained. The parasites were present in the liver. A year later they obtained a strain of *P. falciparum*, infected 770 mosquitoes and inoculated another human volunteer. About 6 days later a liver biopsy was taken and again the parasite was found.

**Pyrotherapy**

In 1927 Julius Wagner-Jauregg won the Nobel prize for his discovery of malaria pyrotherapy for treatment of late stage neurosyphilis. To induce repeated spikes of high fever in patients with progressive paralysis, he inoculated them with blood from patients who were suffering from tertian malaria (*Plasmodium vivax*). Although not without risk, this treatment proved to be very successful.

**Life cycle**

When a mosquito lands on the skin, it attempts to pierce a small blood vessel with its proboscis in order to suck blood. To prevent the blood from coagulating the mosquito first injects some saliva. Besides vasodilating agents this saliva contains anticoagulants. However, the saliva may also contain micro-organisms. When a human is bitten by an Anopheles infected with malaria, parasites (sporozoites) [Gr. sporos = seed] are introduced into the human body. On average 10-20 sporozoites are injected per bite, although this number can be higher, e.g. 100.

A certain protein of the parasite, (the circumsporozoite protein, CSP), plays an important role in the penetration of the sporozoite into a liver cell (cf. Mosquirix vaccine). Sporozoites reproduce asexually in liver cells, by schizogony [Gr. schizo = split, divided]. This is called exo-erythrocytic or pre-erythrocytic reproduction. The form of the parasite produced in this way is called a liver schizont. The multinuclear schizont splits into many thousands of small offspring (merozoites) [Gr. meros = part]. Every successful sporozoite can produce some 20,000 merozoites.

After some time the infected liver cells burst and the merozoites enter the blood stream. While the parasites are reproducing in the liver, there are no symptoms. Neither the sporozoites, nor the liver forms are sensitive to most of the drugs used in malaria prophylaxis (atovaquone/proguanil is an exception). The minimal required time from infection to the appearance of the first merozoites, is the prepatent period. The incubation period is somewhat long because signs and symptoms do not appear until the parasitaemia is sufficiently advanced. Of note, merozoites in blood are usually too
small to be seen by microscopy.

In the case of *P. vivax* and *P. ovale* only some of the infected liver cells burst. The parasites in the liver cells which do not burst (hypnozoites) [Gr. hypnos = sleep] may remain viable for years and are responsible for new attacks of the disease if reactivated. The trigger which reactivates the hypnozoites is not known. The existence of hypnozoites in *P. vivax* was only formally demonstrated in 1985 via fluorescence microscopy. Reactivation of these “sleeping” forms explains delayed exacerbations of the disease after treatment with chloroquine and other antimalarial drugs. They kill the blood forms, but not the liver forms. Hypnozoites are not present in *P. falciparum* and probably not in *P. malariae* (although this is controversial). This is important for treatment because hypnozoites are not sensitive to chloroquine, quinine, mefloquine or artemisinin. Accidental inoculation with infected blood (blood containing trophozoites) may lead to infection, e.g. transfusion malaria or malaria via shared contaminated syringes by drug users. Since the infection in these cases is not transmitted by sporozoites, there are no liver forms. Liver forms are also absent in congenital malaria. This is important for treatment (no primaquine for congenital malaria with *P. vivax* or *P. ovale*). The chronic nature of infections with *P. malariae* is traditionally explained by assuming that the parasite can induce a very low parasitaemia (or hidden erythocytic schizontes) for many years, which is below the detection threshold of normal diagnostic methods.

In the red blood cell the parasite feeds on haemoglobin. The form of the parasite when present in the red blood cell is now known as a trophozoite (Gr. trophe = nutrition). The young parasite possesses a digestive vacuole with lysosomal enzymes. This vacuole contains proteinases (plasmepsin and falcipain). The vacuole can be clearly seen in a blood smear and explains the ring shape of the young parasite. The breakdown of haemoglobin results in an iron-containing pigment: hemozoin. The vacuole disappears as the parasite becomes older. The trophozoites will once more reproduce asexually and lead to the formation of a multinuclear parasite (schizont). The latter divides to form merozoites. Each schizont produces 8 to 24 merozoites, depending on the species, within a time span of 48 hours (*P. falciparum, P. vivax, P. ovale*), 72 hours (*P. malariae*) or 24 hours (*P. knowlesi*). The infected red blood cells burst after a while so that once more merozoites appear in the blood from where they will penetrate new erythrocytes within a few seconds. This bursting (lysis) of the red blood cells is accompanied by a bout of fever. If the development is synchronous (all parasites being at the same stage of development) the fever will follow a typical pattern (see below). This is, however, unusual: asynchrony is more common than synchrony, especially early in infections. The development from merozoite to schizont takes place in the peripheral blood and all stages can be observed. In *P. falciparum* usually only very young forms (ring forms) can be observed in the peripheral blood because older parasites (and schizonts) adhere to the endothelium of blood vessels in deep organs (e.g. the brain).
After a few days some of the merozoites transform into male or female gametocytes. These are necessary for sexual reproduction of the parasite (which only occurs in the mosquito). Gametocytes are responsible for transmitting the disease but do not themselves cause symptoms. Adult *P. falciparum* gametocytes are not sensitive to chloroquine and quinine, while those of *P. vivax*, *P. ovale* and *P. malariae* are sensitive. This means that following adequate treatment of *P. falciparum* there may still be gametocytes in the blood, and this may continue for several weeks. This does not mean that the treatment has failed. One interesting hypothesis is that chloroquine might significantly increase the gametocytemia of chloroquine-resistant *P. falciparum*, resulting in an increased infectivity for *Anopheles*. This could, therefore, contribute to the rapid spread of chloroquine resistance.
**Glucose metabolism and LDH**

The trophozoite has no carbohydrate reserves and needs to consume glucose continually. The glucose metabolism in infected red blood cells is 50-100 times higher than that in non-infected cells. This probably contributes to the hypoglycaemia which is often seen in severe infections.

The parasite have mitochondria, but these play a minor role in the provision of energy (the last word on this has not yet been said). Glucose is converted by anaerobic glycolysis to pyruvate and then to lactate. This latter step, as in humans, is catalyzed by the enzyme lactate dehydrogenase (LDH). The parasite’s LDH is clearly different from that of humans and forms the basis of a diagnostic test (see below).

**Geographical distribution**

Many lay people regard malaria as a purely tropical disease. However, the distribution of malaria used to be world-wide. Today, it still occurs in some 100 countries. The situation varies from region to region. Until 1938 there was still *P. vivax* malaria (“polderkoorts”) in Belgium, and in the Netherlands as late as 1958, although there was an unexplained (possibly autochthonous) case of *P. malariae* infection in a child in Zeeland in 1969. The WHO declared the Netherlands officially malaria-free only in 1970. It is chiefly the pollution of surface waters which makes reproduction of *Anopheles* mosquitoes difficult.

Yet some *Anopheles* persist and can transmit malaria. *Anopheles atroparvus* is able to transmit *Plasmodium vivax* malaria but cannot transmit *Plasmodium falciparum*. *Anopheles plumbeus* can transmit tropical falciparum malaria. In the last century there were important changes in the lifestyle of humans, resulting in less human/mosquito contact. Effective therapy was available. All these factors mean that malaria has disappeared in Northwest Europe. Cases in Western countries are generally dealt with swiftly and satisfactorily and one person with malaria very rarely leads to the infection of others. Chronic large scale reintroduction of the disease in Europe is thus improbable, although with the combination of the current economic crisis with its plummeting health budgets, the massive influx of tropical migrants refugees and global climate change, makes this possibility more real at present than in the last decades of the 20th Century. To maintain an infectious disease, it is necessary for one infectious case to lead to one other infectious case, otherwise the disease will die out in the area. One would need sufficient gametocyte carriers and vectors to ensure the continuation of the disease.

Malaria is a very important public health problem in most tropical countries although the incidence
rate of malaria declined globally between 2010 and 2018 from 71 to 57 cases per 1000 population at risk. In 2018, an estimated 228 million cases of malaria occurred worldwide, compared with 251 million cases in 2010. In that same year 405,000 people died of malaria mainly young children in Africa. Most lethal infections are due to Plasmodium falciparum. Six countries cause more than half of all malaria cases worldwide: Nigeria (25%), DRC (12%), Ivory Coast, Mozambique and Niger (4% each). Of note, the decrease in incidence seems to stagnate these very last few years in sub-Saharan Africa.

P. falciparum is the most common form in sub-Saharan Africa (99.7% of malaria cases in this region), tropical South America and Southeast Asia. The parasite occurred previously in the Mediterranean basin.

P. vivax has the widest distribution area (previously as far as London, Norway, Denmark, New York, southern Canada and even Siberia). In 1922 the number of cases in Texas was estimated at 500,000. It is the most common form in certain regions (e.g. Maghreb, Middle East countries, parts of China, Argentina). P. vivax preferentially penetrates young red blood cells (reticulocytes). In 1976 Miller discovered that P. vivax uses the Duffy blood group antigens (Fya and Fyb) as receptors to penetrate red blood cells. People who do not have this protein on their red blood cells cannot be infected with P. vivax. These antigens do not occur in the majority of humans in West Africa [phenotype Fy (a-b-)]. As a result, P. vivax does not occur in West Africa, or occurs in low numbers (and could be systematically missed). Duffy blood group negative erythrocytes are, in vitro, also resistant to infection with P. knowlesi (monkey malaria).

P. ovale is found chiefly West Africa, less elsewhere in Africa and sporadically in the Far East.

P. malariae is not very common but can be found anywhere. Often confused with P. knowlesi.

P. knowlesi is known from Malaysia (including Borneo), The Philippines, Vietnam, Thailand and recently Myanmar. The vector is present in India (Kerala) and Sri Lanka, but in these areas there is no known zoonotic reservoir. P. knowlesi infections are often confused with P. malariae.

Malaria can only persist naturally when climatic conditions are suitable for the vector(s) and for the development of the parasites in the vector. Increased rainfall and higher temperatures may make larger areas favourable for malaria transmission in the future. Tropical P. falciparum requires a minimum temperature of 18°C, while tropical P. vivax strains require a minimum of 16°C. The European strain of P. vivax which persisted in the high North was uniquely adapted, with summer temperatures being sufficiently high for P. vivax development in the mosquito. Infection of patients
occurred in autumn (September / October) when mosquitoes started to enter homes looking for shelter. The P. vivax parasites in humans had a very long incubation period of 6 to 9 months. A patient infected with these northern strains of P. vivax would remain asymptomatic during winter until the following spring. This clearly differs from tropical P. vivax dynamics. In Southern Europe P. falciparum used to be common in Portugal, Spain, Italy and Greece. It is likely that the strain of this parasite was genetically different from tropical strains.

However for malaria to become re-established, a sizable parasite reservoir (gametocyt carriers) must be present. This has not happened in other circumstances, such as after World War II, when great numbers of people (patients and gametocyt carriers) returned from tropical areas. In the current health system and socioeconomic situation in Europe, it is likely that patients will be treated early, diminishing the reservoir and lessening the threat of new epidemics. Small outbreaks and some local transmission might occur from time to time, but large reinvasion of the North European landmass is unlikely. South Europe would have a somewhat larger risk, as reflected by the outbreak of autochthonous P. vivax cases in Greece in 2012.

**Epidemiological classification - stable versus unstable malaria**

There is no completely satisfactory epidemiological classification of malaria. Stable malaria means that the clinical disease is characterized by preferentially affecting children and achieving a protective “immunity” in adults. Stability does not mean that there can be no variation in transmission. In some regions seasonal malaria occurs. In other areas there is unstable malaria: transmission differs greatly from year to year and occasionally epidemics occur. Then the disease also occurs in older persons. This is important in many respects; as irregular control of malaria may lead to changes in the immune status of the population. Sometimes malaria may appear again in a region after a long absence. For example: in 1972 the disease was eradicated in South Korea following an intensive eradication campaign with case detection and vector control. In 1993 one case of P. vivax was observed. There then followed 22 cases in 1994, in 1995 there were 107 cases, 356 in 1996 and more than 1600 in 1997. In 1995 all cases were still limited to the border area with North Korea, but in 1996 there was also transmission outside the demilitarized zone. After entomological surveys had shown that *Anopheles sinensis* was the chief vector, measures were taken to control the disease.

**Vector, Anopheles mosquitoes**
Malaria is transmitted via the bite of infected female Anopheles mosquitoes.

Malaria is transmitted by Anopheles mosquitoes. This applies to the malaria of all mammals. Avian malaria is chiefly transmitted by Culicinae. There are some 400 Anopheles species, 40 of which are good vectors while 28 are poor vectors. Anopheles mosquitoes are active at night. They do not buzz much and are not easily noticed. The world’s most important vector is Anopheles gambiae, an anthropophagic and endophilic freshwater mosquito which flourishes preferentially in moist regions. It typically breeds in exposed sunlit and often transient aquatic habitats such as pools, puddles, and irrigation channels. Anopheles mosquitoes are good flyers: they can cover several kilometres in one night. This is of course of great importance for their control. Endophagic (bite inside the house) mosquitoes will often rest on walls after a blood meal. Residual insecticides which are applied there will kill the vector.

How do mosquitoes find their prey?

Mosquitoes are attracted by an increasing CO₂ gradient. The warmth of the skin, lactic acid and moisture (breath) play a part over short distances. Every animal produces several volatile substances in its skin, breath, faeces and urine. A number of the substances (kairomones) are used
by the mosquito to find its prey. The details are complex. Anopheles gambiae prefers to land on the feet, while A. atroparvus prefers to bite the face.

Vector control

Malaria vector control is primarily based on the use of insecticides. Appropriate monitoring of vector resistance to insecticides is an integral component of planning and evaluation of insecticide uses in malaria control programmes. Pyrethroids and DDT, two important insecticides used for vector control, block the nerve-impulse conduction by preventing a sodium channel from closing after an action potential. An important mechanism that confers resistance to pyrethroids and DDT, known as knockdown resistance or kdr, was first described in the housefly Musca domestica. It has been reported that a single mutation in the sodium channel sequence is the molecular basis of kdr in Musca domestica. The gene has also been characterized for Anopheles gambiae. PCR tests have been developed for the detection of the kdr-mutation in A. gambiae.

Physiopathology

The incubation period may be short (minimum 7-9 days for P. falciparum) to very long (several years for P. ovale). In falciparum malaria the parasitaemia can be very high: up to 80% of erythrocytes may contain parasites, but even 5% is enough to result in severe disease. These situations may be life-threatening. The other malaria parasites produce much lower parasitaemia (especially P. ovale). They do cause severe illness but are rarely life-threatening. P. knowlesi infections mimics severe P. malariae infections.

The rupture of the red blood cells (haemolysis) is accompanied by fever, muscle pain and general malaise. Massive haemolysis may cause kidney failure. Parasitized red blood cells are removed by the spleen. Splenomegaly will result. Anaemia occurs due to the destruction of erythrocytes, suppression of the bone marrow and excess activity of the enlarged spleen (hypersplenism). In severe falciparum malaria, there is activation of blood coagulation system along with thrombocytopenia, even before widespread DIC and coagulation failure occur. In falciparum malaria there will often be a drop in glycaemia that can be corrected by administration of glucose.

The details of how cerebral malaria happens, are not clear at present, and various researchers have different opinions. More than 100 years ago, the Italian pathologists Bignami and Marchiafava reported on the sequestration of parasitized red blood cells in the brains of people who died of cerebral malaria. Erythrocytes which contain schizonts of P. falciparum, develop small knobs on their
cell membranes. These consist, among other things, of a histidine-rich protein, *P. falciparum* erythrocyte membrane protein 1 and rifins. Rifins are clonally variant proteins encoded by rif genes (“repetitive interspersed family”) and are expressed at late ring or early trophozoite stage on the infected red cell surface. Their high copy number, sequence variability, and red cell surface location indicate an important role in host-parasite interaction. The knobs have an overall negative charge, allowing non-specific attraction to positive endothelial ligands, but specific molecular adhesion also play a part. With these knobs the infected cells cling to the walls of the capillaries and to the vascular endothelium of the post-capillary venules in the brain. The low local O2 pressure and high CO2 pressure are optimal for further maturation of the parasite. Infected red blood cells are less easily distorted and more rigid than normal erythrocytes. This impedes the blood flow, which can lead to cerebral malaria. Other organs may also be affected for example the placenta and the intestines (resulting in abdominal pain and diarrhoea). Red blood cells which contain schizonts of *P. malariae*, also develop knobs on their membranes, but these cells do not adhere to the vascular endothelium. When post mortem cerebral sequestration was compared with the peripheral parasitaemia, there were about 26 times more infected red blood cells in the brain microvasculature than in the peripheral blood if there were free-mixing. More blood vessels in the cortex and cerebellum than in the brain stem are affected. Some researchers found more sequestration in white matter than in cortex. Coma requires sequestration, but sequestration itself is not enough to provoke cerebral malaria. The rapid reversible nature of cerebral malaria led to the hypothesis that soluble neuroactive mediators might play a role in the pathogenesis possibly involving reversible disturbances of the blood brain barrier and biochemical disruption of normal metabolism.

There are two groups of parasites in *P. falciparum* infections: (1) the young forms in the peripheral blood which can easily be observed in a thin blood smear and (2) the mature group which is attached to small blood vessels and which cannot be seen. Falciparum schizonts are rarely found in peripheral blood but these are important for the development of cerebral malaria. The whole mechanism of cerebral malaria has not to date been fully explained. As well as the attachment of parasitized red blood cells to the vessel walls (cytoadherence) other mechanisms possibly also play a part. Normal red blood cells sometimes attach to parasitized cells, which impairs the microcirculation. All kinds of released chemical substances (cytokines, oxygen radicals, etc.) may also play a part. Cytokines such as tumour necrosis factor (TNF-α) increase the expression of receptor molecules on the endothelium and will contribute to the cytoadherence and flow obstruction which characterize falciparum malaria. This mechanism is similar to the release of TNF-α by endotoxins in Gram-negative septicaemia.

Increased brain volume was seen in children who died from cerebral malaria but was uncommon in those who did not die from the disease; this suggests that raised intracranial pressure may contribute to a fatal outcome.
Carriers of the sickle cell anaemia gene (heterozygotes for haemoglobin S) have relative protection against severe infection with \textit{P. falciparum} and thus have a survival advantage (in homozygous patients, malaria may be fatal and the disease itself tends to kill patients before the reproductive age). The same advantage probably applies to persons deficient in G6PD. This may explain why these two conditions are so common in Africa. In Papua New Guinea ovalocytosis is common. These red blood cells have an oval shape and cannot be penetrated by \textit{P. falciparum} parasites. Heterozygotes are thus protected against \textit{P. falciparum} (homozygosity is not compatible with life).

In West Africa, haemoglobin C is rather frequent. People with haemoglobin AC or CC can be infected with \textit{Plasmodium falciparum} and can develop substantial parasitaemia. The presence of Hb C therefore does not protect against infection itself. Haemoglobin C might protect against the lethal effects of \textit{P. falciparum} malaria by reducing cytoadherence of parasitized erythrocytes.

Haemoglobin E (chiefly Southeast Asia) does not protect against \textit{P. falciparum} infections itself.

While circulating in human blood \textit{P. falciparum} exhibits antigenic variation. On the surface of the infected red blood cell a certain protein is expressed: the \textit{P. falciparum} erythrocyte membrane protein 1 (PfEMP-1). The parasite can make many variants of this protein. By interchanging which variant of PfEMP-1 is present, the parasite can evade the immune response to these immune dominant antigens. These proteins are thought to be the major virulence factor found on the surface of infected red blood cells, directly contributing to the pathogenic nature of the infection and placing these genes at the centre of a disease responsible for several million deaths in developing countries. Although there are many var gene copies, only a single var gene is expressed at any given moment (i.e. there is mutually exclusive expression). Over the course of an infection, expression switches from one var gene to another, resulting in antigenic variation of the parasite population and a persistent infection which is difficult to clear by the human immune system.

Antigenic variation has important implications for the development of vaccines. The repertoire of proteins which are expressed in the \textit{Anopheles} mosquito is far less pronounced probably because the vector has no adaptive immune system.

A large case-control study of malaria in West African children showed that a human leukocyte class I antigen (HLA-Bw53) and an HLA class II haplotype (DRB1*1302-DQB1*0501), common in West Africans but rare in other racial groups, are independently associated with protection from severe malaria. In this population they account for as great a reduction in disease incidence as sickle-cell trait. These data support the hypothesis that the extraordinary polymorphism of major histocompatibility complex genes as well as other genes has evolved primarily through natural
selection by infectious pathogens.

Malaria is very often accompanied by thrombocytopenia, the causes of which seem to be multiple and not completely known. The severity of the thrombocytopenia correlates with the parasitaemia and the clinical severity of infection.

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Malaria - Clinical aspects

Classic acute uncomplicated attack

Most clinical episodes of malaria are characterized by fever with aspecific symptoms. Certainly, in children the presentation can be very misleading. Any fever should bring the possibility of malaria to mind. There is a danger, however, that every fever episode may be regarded as malaria and other important diagnoses are then likely to be missed.

*P. falciparum*: typical incubation time: 7 to 30 days. If a person is taking preventive antimalarials and if the parasite is partially resistant, there may be temporary suppression of a malaria attack. The fever is generally irregular. If the attack is not treated, after a few weeks a regular fever pattern will develop with peaks every 2 days (tertian malaria, so called because the fever reappears on the third day, reckoning the day of the paroxysm as the first. This is rare in everyday clinical practice). At the beginning of the attack the symptoms are similar to influenza: general malaise, tiredness, muscle pain, headache but in general without respiratory tract problems or running nose. These symptoms are non-specific. After a while the muscle pain and headache become worse. Sometimes there is also abdominal pain and diarrhoea. Rarely there is a classic attack: this lasts for approximately 12 hours and occurs every 48 hours. At first cold shivers with high fever occur, followed by an intense feeling of heat and fever, leading to a sweating stage with a drop in fever. Most falciparum attacks do not follow this classic pattern. Therefore what is referred to as a classic attack is paradoxically not the general rule.

*P. vivax* and *P. ovale*: the incubation time is a few weeks to years. The awakening of dormant parasites in the liver (hypnozoites) explains the potential for late relapses. The fever is sometimes regular (every 48 hours) especially in cases of recrudescence (tertian malaria). In 1922 *P. vivax* was
introduced for the treatment of neurosyphilis. It was thought that the bacterium which causes syphilis had little resistance to heat, so the high fever would kill the bacteria (*Treponema pallidum*).

*P. malariae*: the incubation time is 3 weeks to many years. The very late attacks are probably not due to awakened hypnozoites (to date these have never been detected) but due to the activation of blood parasites which are present at a very low concentration. Fever peaks may occur every 72 hours (quartan malaria).

*P. knowlesi* is a monkey parasite which can be misidentified as *P. falciparum* in the early ring stage and as *P. malariae* in the older stages. It has the shortest asexual life cycle of all i.e. 24h. The prepatent period is 9-12 days. At present, no hypnozoites have been found. PCR is needed to firmly identify this species.

Mixed infections: mixed infections do occur, but for reasons which are unclear they are much less common than would be expected based on the prevalence of the individual species. Underreporting may play a part, but this is probably a real phenomenon (partial cross-immunity to heterologous species? biological interference?).

**Natural course of malaria in the autochthonous population**

Children are very susceptible to infection. The highest mortality is found in children below the age of 5 years. Gradually, after repeated infections, a partial immunity develops in those who survive. There is a high degree of tolerance to the infection in adults, provided they live in a stable malaria region. This semi-immunity (or “premunition”) is maintained by repeated infections and mild latent infections. It disappears after approximately 6 to 24 months if there is no further infection (e.g. a stay in a non-malaria region).

This partial immunity is reduced during pregnancy. A pregnant woman is at increased risk of hypoglycaemia and cerebral malaria. Malaria is an important cause of severe (sometimes spectacular) anaemia in the mother, low birth weight, premature birth, abortion and increased perinatal death. Chondroitin sulphate and hyaluronic acid, both present in abundance around the syncytiotrophoblasts of the placenta, are mucopolysaccharides (glycosamine glycanes) which act as receptors for red blood cells infected with *P. falciparum*. Probably there are also other receptor molecules. Infected cells accumulate in the placenta resulting in reduced placental function.

The placental barrier is very seldom passed. Congenital malaria is not common and occurs chiefly in
neonates of non-immune women. Neonates of semi-immune women receive transplacental anti-plasmodium antibodies. Due to this passive resistance in the first 3-6 months they are at a lower risk of malaria.

Several observations of humans infected with both malaria and helminths suggest that co-infection provides a benefit to either parasite. The evidence indicates that malaria patients co-infected with helminths are protected from severe malaria possibly through skewering of the immune response towards T helper (Th)2 immunity.

Malaria and HIV interact in several, rather complex ways. The CD4+ lymphocytes play a central role in the defence against malaria and their characteristic decrease during the course of HIV infection explains why severely immunosuppressed HIV-positive individuals are so susceptible to severe malaria. In malaria-endemic regions, severe malaria may be considered as an “opportunistic infection” and any diagnosis of complicated malaria in adults should trigger HIV testing.

**Acute severe malaria**

Acute severe falciparum malaria is a medical emergency. This encompasses:

- Coma: repeated generalized convulsions
- Hypoglycaemia: reduced consciousness, aggressive behaviour
- Severe anaemia: weakness, tachypnoea, pale mucosae
- Tendency to spontaneous bleeding (pronounced thrombocytopenia)
- Circulatory collapse (shock); cfr. below “algid malaria”
- Pulmonary oedema (dyspnoea and bilateral crackles) leading to acute respiratory distress syndrome (ARDS)
- Haemoglobinuria (dark urine)
- Kidney failure: urinary output should be monitored (but attempts to force urine production may cause circulatory overload!)
- Acidosis (chiefly due to lactic acid): tachypnoea. If too many salicylates are given this may exacerbate acidosis (not unusual in febrile patients).
- Other important signs are: marked jaundice, confusion without coma, extreme generalized weakness or prostration (child cannot remain sit and don’t want to eat/drink).

The priorities are cerebral involvement, severe anaemia, hypoglycaemia, and the presence of hyperparasitaemia. The degree of parasitaemia correlates with the severity of the symptoms: the higher the parasitaemia, the greater the risk of severe symptoms. It should be borne in mind that the
parasitaemia (the percentage of parasitized cells that are found in a smear preparation) changes by the hour. This is because the red blood cells with mature *P. falciparum* parasites (schizonts) attach themselves to the small capillaries of deep organs therefore are not found in a thin blood smear. A parasitaemia of 0.5% is already severe, 2% is pronounced, and patients with a parasitaemia of more than 10% have a relatively poor prognosis. Over 25% is often fatal. Another consideration is that a parasitaemia of 3% in someone who still has a normal red blood cell count, is different from a parasitaemia of 3% in an anaemic patient.

**Hypoglycaemia** may quickly lead to general deterioration and coma. It is common in children (up to 25% of cases) and pregnant women. Glucose may be life-saving. If the glycogen store in the liver is low (i.e due to malnutrition) the risk of hypoglycaemia increases [glycogen is converted to glucose]. The conversion of glycogen to glucose is also inhibited by certain cytokines which are released during infection with *P. falciparum* [Hypoglycaemic effects of TNF-a and possibly interleukin-1 and TNF-β]. The parasites themselves also use glucose for their metabolism and contribute to the hypoglycaemia if they are present in large numbers. Quinine can stimulate the secretion of insulin from the pancreas and in this way can also contribute to hypoglycaemia.

The term **“algid malaria”** (L. “algidus” = cold) is obsolete. The condition is characterized by hypotension with progression to shock. The patient is clammy and often feels cold. There is no fever. Often there is concurrent septicaemia with Gram-negative bacteria. Mortality is high. Therapy with artesunate or quinine, treatment with antibiotics and (cautious, see ARDS) IV fluid administration is of great importance. Shock seldom occurs in malaria if there is no septicaemia. However splenic rupture can also cause hypovolemic shock.

**Splenectomy.** This may occur spontaneously or after an unobserved trauma. This complication can occur in *P. falciparum, P. vivax, P. ovale or P. malariae*. The presence of intraperitoneal fluid is suggestive in this context. In these cases ultrasound can often detect a splenic hematoma, splenic rupture or intraperitoneal fluid. A diagnostic peritoneal lavage may be indicated.

**Cerebral malaria** is the main cause of death (80 %) in falciparum malaria. This complication occurs chiefly in non-immune persons (children, travellers). Cerebral signs include confused behaviour, psychosis, convulsions, stupor, coma, paralysis. Unlike meningitis, there is no real neck stiffness (pain) or photophobia (intolerance to light) but neck retraction and opisthotonos (neck muscle spasm) may occur. Sometimes the difference between neck stiffness and neck retraction is not clinically clear. It is typical of the coma that it develops swiftly in 75% of cases and quickly disappears. If a child survives cerebral malaria it has approximately a 10% chance of significant long term sequelae. Children with cerebral malaria and with a normal eye fundus have a good prognosis, while
papilledema and retinal bleeding suggest a guarded prognosis. Malarial retinopathy is increasingly considered as a specific diagnostic criteria of cerebral malaria, but sensitivity of this abnormality is rather poor (meaning that its absence does not exclude cerebral involvement). Repeated generalized convulsions should not be regarded as “normal” febrile convulsions. Severe convulsions with contraction of the abdominal muscles and compression of the stomach, may cause reflux of gastric acid and food into the pharynx. Aspiration of gastric contents into the lungs is a real danger as this may result in Mendelson’s syndrome (chemical pneumonitis) or aspiration pneumonia. If there are convulsions, these are stopped by administering diazepam (Valium®) IV. A CT scan or MRI scan of the brain of patients with cerebral malaria shows few abnormalities except an occasional increase in cerebral volume. Herniation of the brain stem is a rare event.

If confronted by a febrile coma or confusion with fever in the tropics, glucose must be administered (preferably IV), artemisinin (or quinine if artemisinin unavailable) therapy should be instituted and a lumbar puncture carried out without hesitation (to rule out meningitis). Of the persons who die in hospital due to cerebral malaria, 50% of the fatalities occur within the first 12 hours after admission. At autopsy countless petechiae can be seen in the brain. Small ring-shaped haemorrhages also occur around cerebral blood vessels.

**Febrile convulsions**

Febrile convulsions are generalized tonic-clonic convulsions. They only occur in children between the age of 6 months and 5 years and will not be repeated during the same fever episode. They occur during the phase in which the fever is rising fast. They always last less than 15 minutes, but post-ictal coma can take up to 1 hour. There is never postictal hemiparesis. It is important to differentiate between febrile convulsions and convulsions during fever (e.g. cerebral malaria, meningitis, cerebral abscess). Approximately 2% of children have a tendency (possibly genetic) for febrile convulsions. The risk that epilepsy will develop in this group of patients is no greater than in children without febrile convulsions. Brief and sporadic attacks have a good prognosis. No maintenance therapy with anti-epileptic agents is required.

**Severe anaemia** occurs due to haemolysis (of both parasitized and non-parasitized red blood cells – the latter via immune-mediated mechanisms), due to excessive action of the spleen i.e. hypersplenism (until weeks after the infection), due to possible haemorrhages (low blood platelets, splenic rupture) and due to disturbed production of new blood cells in the bone marrow (dyserythropoiesis) due to TNF-alpha.
Hyperpyrexia (very high fever above 40°C) should be treated by cooling the patient and administering paracetamol. It is assumed that malaria fever is caused when lysis of the red blood cells releases malaria pigment (hemozoin) as well as GPI-anchors ("malaria toxin") which are absorbed by the reticulo-endothelial system. This in turn releases endogenous pyrogens (cytokine network). The concentration of tumour necrosis factor in the peripheral blood correlates with the severity of the malaria. In cases of repeated malaria attacks the liver, spleen and bone marrow are stained black by the enormous amounts of hemozoin. Hyperpyrexia is no longer considered as a criteria of severity (WHO classification of 2000).

Black water fever

Black water fever is a severe life-threatening complication. Acute massive haemolysis occurs through immuno-allergic mechanisms which are not fully understood. It has been observed after taking halofantrine, artemisinin-derivatives and after irregular use of quinine. The parasitaemia is generally very low. There is high fever, jaundice, back pain, shock and very dark urine. Renal insufficiency occurs: the urine production is very low (oliguria) or zero (anuria). Mortality is very high. When quinine was no longer used prophylactically, black water fever became very rare. Differential diagnosis should be made with severe malaria itself, leptospirosis and viral haemorrhagic fever.
Acute renal failure may also be caused by shock, hypovolemia with reduced renal circulation, disseminated intravascular coagulation (DIC), obstruction of the renal glomeruli by parasitized red blood cells and by the precipitation of released haemoglobin in the kidney (pigment nephropathy). The combination of these factors can result in acute tubular necrosis. Glomerulonephritis may occur in chronic *P. malariae* malaria (cfr. infra), but this complication plays no part in acute renal problems.

**Pulmonary oedema** is a common complication of severe malaria. The dividing line between overhydration and dehydration is narrow. Adults easily develop non-cardiogenic pulmonary oedema if there is excessive fluid overload, but on the other hand dehydration and hypovolemia may lead to hypotension, shock and renal failure. Pneumonia is observed quite often if coma lasts for longer than 3 days. ARDS (acute respiratory distress syndrome) may occur. This is caused by diffuse damage to the vascular endothelium and the alveolar epithelium. There is a rapid progression towards dyspnoea, arterial hypoxia, bilateral patchy pulmonary infiltrates due to pulmonary oedema with a protein-rich...
fluid. The treatment is both etiological and symptomatic: mechanical ventilation, with or without intubation or an endotracheal cannula, possibly with NO (nitrogen monoxide), high-dosed oxygen and positive end-expiratory pressure (PEEP).

**Chronic falciparum malaria**

Where *P. falciparum* is partially resistant to the therapeutic drug locally used (e.g. chloroquine), the parasite may be suppressed, but will remain present (not completely cleared). This may lead to a whole range of clinical pictures, from asymptomatic parasitaemia through to mild aspecific symptoms, to significant chronic malaise, anaemia and fatigue. Curative therapy with atovaquone/proguanil or artemisinin-based combination therapy (ACT, see below), for example, produces rapid improvement.

**Hyperreactive malaria splenomegaly (HMS)**

Some adults have a very strong immunological reaction to *P. falciparum* antigens. The level of IgM in the blood is very high. Due to the polyclonal immune stimulation, all kinds of autoantibodies can appear. Immune complexes are formed, and are removed by the reticulo-endothelial system, which leads to splenomegaly and sometimes hepatomegaly. In these individuals the swollen spleen swells also breaks down normal, non-parasitized red blood cells. The number of parasites is very low, but very high concentrations of anti-*P. falciparum* antibodies can be detected. The splenomegaly disappears after curative therapy with, e.g. ACT followed by months or even years of adequate malaria chemoprophylaxis if persistent exposure in a malaria region), but recovery is very slow. In rare cases splenectomy is necessary. Steroids have no place in the treatment.

**HMS and splenic lymphoma**

HMS may be very similar to a certain indolent splenic lymphoma (e.g. splenic lymphoma with villous lymphocytes). The latter disorder is related to B-cell chronic lymphocytic leukaemia and occurs chiefly in elderly persons. The disease is often accompanied by significant cytogenetic abnormalities and monoclonal “villous” B-lymphocytes in the peripheral blood. It is likely that in HMS, excessive stimulation of the B-lymphocytes by malaria antigens increases the risk that oncogenic mutation may occur, followed by clonal growth of these cells.

**Burkitt’s lymphoma**

This malignant tumour originates from B-lymphocytes. It is very aggressive with a volume doubling time of about 3 days. The endemic form occurs in sub-Saharan Africa and is also found in Papua New
Guinea. In these areas, it accounts for up to 50% of childhood tumours.

One hypothesis states that repeated malaria attacks may have a mitogenic effect on infected B-lymphocytes (polyclonal B-cell stimulation) increasing the risk of mistakes during chromosomal replication which subsequently would lead to neoplastic behaviour.

Burkitt’s lymphoma generally presents with swelling of the jaw and mouth ulcerations (75%, especially maxilla tumours), abdominal swelling with ascites (60%) and central nervous system involvement (30%, including cranial nerve palsies, malignant pleocytosis or paraplegia). Infection with the Epstein-Barr virus (cf. mononucleosis) plays an important part in the endemic form of Burkitt’s lymphoma, probably by causing genetic instability. Epstein-Barr viral DNA is found in about 90% of African Burkitt’s lymphomas.

The tumour responds well to cytostatic drugs. The alkylating agent cyclophosphamide (Endoxan®) is first choice (the target dose 1-1.5 gram/m² IV every 3-4 weeks with 2 doses in remission), but more complex chemotherapies (methotrexate, vincristine, CHOP-R, hyper-CVAD,...) are difficult to evaluate in low-resource settings. About 80% of patients can achieve complete tumour regression and 10% have a partial response. About 50% will relapse.
Nephrotic syndrome secondary to chronic infection with Plasmodium malariae. Notice the swollen face and ascites. Photo prof. Gigase. Copyright ITM

Burkitt’s lymphoma in a Cambodian woman, aspect before chemotherapy. Photo Dr Lut Lynen, copyright ITM

**Nephrotic syndrome in P. malariae**

Chronic infection with *P. malariae* may, via immunological mechanisms (chronic immune complex glomerulonephritis) cause a nephrotic syndrome, characterized by oedema and proteinuria (more than 3.5 gram per 24 hours). There is often significant hyperlipidaemia and lipid bodies are sometimes found in the urine.

If a kidney biopsy is carried out, it should be borne in mind that severe bleeding will occur in 1% of cases. The treatment of nephrotic syndrome is difficult. Curative malaria treatment is of course
indicated but will not produce improvement of the kidney function. Salt restriction and diuretics are indicated (both thiazide and loop diuretics). Treatment with an ACE-inhibitor [angiotensin-converting enzyme-inhibitor such as enalapril] should be ideally initiated in settings where it is available. Steroids and immunosuppressive agents are of little benefit in this disorder. An important challenge is to distinguish the entity from minimal change glomerulonephritis (electron microscopy needed to confirm “minimal change” on biopsy specimen).

Malaria - Diagnosis

General

When can one assert that someone has the disease “malaria”? There are several problems and the question has still not been fully resolved. The demonstration of malaria parasites in the blood is essential but insufficient in itself. Most cases are accompanied by thrombocytopenia and normal white count. Many people will develop an acquired immunity after several years of exposure and may harbour parasites without exhibiting symptoms. The degree of parasitaemia may help, but there is no absolute criterion (the higher the parasitaemia, the more chance that malaria is in fact the diagnosis). There are patients with malaria for whom the thick smear is negative (luckily this is rare in a good laboratory). There are no pathognomonic clinical signs. An accurate diagnosis is becoming more and more important, in view of the increasing resistance of \textit{P. falciparum} and the higher price of modern combination treatments.

Clinical aspects

No single clinical sign allows the diagnosis of malaria. Most cases are accompanied by thrombocytopenia, a normal white count and a positive parasitaemia. Yet malaria must always be considered in cases of fever in the tropics. Since the symptoms can be quite diverse, a clinical diagnosis is unreliable and the diagnosis should be based on identification of the parasite. Microscopic confirmation of the diagnosis is often not possible in many regions and situations. It is of the greatest importance that other important diagnoses are ruled out before instituting a blind anti-malaria therapy. All too often fever is considered as malaria without considering alternative diagnoses. This tendency is reflected in the quote: “if you only have a hammer, you tend to see every problem as a
nail” (Abraham Maslow).

The presence of parasites does not rule out an additional diagnosis: e.g. someone with fever may well have some malaria parasites in a thick smear, but this does not rule out meningitis or pyelonephritis. Chronic carriers are people who, although they have malaria parasites in their blood, have no symptoms of this. When such people develop another infection their symptoms are often attributed to the malaria parasites in their blood, although these are not responsible. The absence of parasites in a single preparation does not rule out malaria but does make the diagnosis of *P. falciparum* highly improbable (if the microscopist searched carefully). Where there is any clinical suspicion it is best to repeat the test 12h later.

**Microscopy**

A thick smear concentrates the parasites 10 to 25 times. It is rather more difficult to interpret than a thin smear preparation and often does not permit species identification. A thick smear contains no intact red blood cells (haemolysis due to the distilled water used in the staining). If a thick smear is positive, a thin smear should be examined.

**Parasitaemia**

The parasitic density can also be roughly determined in a thick smear, by counting the number of parasites per 200 leukocytes and multiplying this by 30. It is assumed that on average there are 6000 leukocytes per µl blood and that there is one leukocyte per 500 red blood cells. For example: 5 parasites per leukocyte (1000 parasites for every 200 leukocytes) corresponds to a density of 30,000 parasites per µl. Roughly 30,000 parasites per µl corresponds to a parasitaemia of 1% (5 parasites per 500 RBC’s): a moderately anaemic person.

If the thick smear is found to be negative in a reliable laboratory and if there is strong suspicion of malaria, the test is repeated every 12 hours for 48 hours. One great disadvantage of the thick smear method is that reliable technical expertise is needed which should be monitored (e.g. quality control). The argument that a lab technician has carried out the test for years and thus has plenty of experience is absolutely no guarantee of quality or reliability. The test also requires plenty of time if the parasitaemia is low, or before a negative result can be concluded.

A thin blood film has many advantages:

- it demonstrates the species present
• detection of mixed infections is possible
• distinguish asexual stages from gametocytes
• assesses parasitaemia (in % of infected red blood cells)
• can detect a new or unexpected parasite
• gives information on red cell morphology
• allows a white cell differential count
• inexpensive

Other points include: Sensitivity and specificity is operator dependent. In a good average lab, the sensitivity is good but limited to about 50 parasites per µL, this is somewhat better in a reference lab. Most routine laboratories cannot detect parasitaemia below 100 to 500 parasites per µL. DNA amplification techniques have better sensitivity and can give information when species is in doubt but this technique remains limited to reference laboratories (even in high resource settings).

If the parasite cannot be identified it is regarded as a *P. falciparum* as a safety precaution. Mixed infections do occur.

**Antigen detection**

Malaria rapid diagnostic tests (RDTs) based on lateral-flow immunochromatography are increasingly used in endemic and non-endemic settings. They are easy to use, provide results rapidly and require no specific training and equipment. Reported sensitivities vary between different RDT products but are generally good for *Plasmodium falciparum*, with rapid tests based on the recognition of *P. falciparum* antigen histidine-rich protein-2 (PfHRP2) scoring slightly better than those which recognize *P. falciparum*–lactate dehydrogenase (LDH). Sensitivity is lower for *Plasmodium vivax* (66 – 88%) and usually poor for *Plasmodium ovale* (55 – 85%) and *Plasmodium malariae* (21 – 45%). Rapid diagnostic tests have some limitations. The test strips are susceptible to heat and humidity. A positive result can be obtained after correct treatment, when there are no more parasites visible in the thick blood smear. This is due to persistence of the PfHRP2 antigen (up to several weeks) after successful treatment. The pLDH based tests have the advantage of turning negative sooner after parasite clearance (several days). Occasionally there is cross-reactivity of *P. falciparum* with the non-falciparum test line and vice versa and rare false-positive reactions due to other infectious agents or immunological factors. False-negative results occur in the case of low parasite densities, prozone effect (saturation of binding sites due hyperparasitaemia) or pfhrp2 gene deletions as observed in Pf strains from South America, but also in Mali, DRC and India. The latter two reasons for false negativity are only observed with HRP2-based RDTs. Finally when instructions are not followed (delayed reading, incorrect sample and buffer volumes, not recognizing invalid test results, disregarding faint test lines)
errors in interpretation can occur. **Rapid diagnostic tests do not give information about parasite density.**

### Depolarized light scatter

Automated cell counters, such as certain Cell-Dyn instruments, use 90° depolarized light scatter to distinguish eosinophils from other leukocytes. Eosinophils are normally the only leukocytes that depolarize light. Some automated haematology analyzers display an alert for possible malaria based on the presence of activated monocytes (Coulter Counter), hemozoin containing white blood cells (Cell-Dyn series) and an additional peak in the reticulocyte fraction (Cell-Dyn series). During malaria infection, the parasites consume haemoglobin and produce malaripigment, a form of polymerized haeme. This pigment, also known as hemozoin, is birefringent. When peripheral blood is analyzed by automated flow cytometry, the pigment will cause atypical depolarization of the laser beam that can be recognized in a scatterplot. Although diagnostic accuracy of these features is too low to exclusively rely on these flags for malaria diagnosis, such an alert is especially useful in situations where the initial clinical suspicion of malaria is low (non-endemic setting).

### PCR

At present, in case of doubt, mixed infections, low parasitaemia, forensic questions, suspicion of zoonotic malaria, etc... PCR technology (e.g. multiplex real-time PCR) can give answers to several questions, but is in general slower than the traditional methods since such tests are not performed everyday even in larger centres. However, point-of-care PCR based techniques are being developed and their importance might grow in the future in countries contemplating malaria elimination, especially if this technique can combine detection of multiple infectious agents (multiplex-PCR). The future will learn whether they will have a place in diagnosis even in low-resource settings.

### Serology

Serology can only be carried out in reference hospitals and is of no importance for the individual diagnosis in acute fever. The antibodies are positive from the tenth day therefore at the beginning of the attack they will be negative. The presence of antibodies only shows that there has been contact with the parasite. This does not mean that there is immunity. There will be high titers of antibodies in the tropical hyperreactive malaria splenomegaly. Malaria type IgG antibodies penetrate the placenta and will give the neonate temporary and partial protection against malaria during the first months of life. Antibodies after infection remain positive for a longer time.
Indirect aspects

Signs of haemolysis include yellow serum, dark urine while faeces have a normal colour, elevated indirect bilirubinaemia and low haptoglobin. Often there is thrombocytopenia. Sometimes there is malaria pigment in white blood cells (sign of severity).

Test therapy

In endemic regions fever, muscle pain or even generally feeling unwell are often attributed to “malaria”. An anti-malaria treatment is then instituted, without obtaining confirmation of the diagnosis or often even without considering alternative diseases. The argument given is that such a treatment can do no harm, that the diagnosis of malaria is always probable because the disease is common and that this is a good strategy for first-line care. Each of these arguments can be defended to a certain extent, but in this way often useless and sometimes expensive treatments with potential side effects are administered. In addition, not recognizing and treating other diseases (borreliosis, rickettsiosis, kidney infections, amoebic liver abscess, pneumonia, sepsis and so on) is a daily reality in many tropical regions. The over-diagnosis of malaria often leads to under-diagnosis of other treatable disorders. It is sometimes stated that fever which does not disappear after three days of adequate therapy, is not malaria. This may however not be completely true, in case of drug-resistant malaria (resistance R3, with no decrease in the parasite load during treatment) or co-infection with another pathogen (commonly sepsis).

In face of the increasing resistance to \textit{P. falciparum} parasite and the need of more complex and expensive treatment (ACT), WHO recommends since 2010 the diagnosis of malaria being parasite-based as often as possible either by microscopy or antigen-based RDTs. Ideally no malaria treatment should be provided without confirmation of the diagnosis.

Malaria - Prevention

External agents

Anopheles mosquitoes only bite in the evening and at night. It is possible to protect oneself by wearing protective clothing and using an undamaged mosquito net. Effectiveness is increased by
treating the net with pyrethroids (insecticides) such as permethrin (Permas®, Peripel®), lambda-cyhalothrin (Danger®, Demand CS®, Matador®) or deltamethrin (K-Otrine®). This will increase further in importance in the future. In most instances, permethrin will be augmented by piperonyl butoxide. Piperonyl butoxide is the most widely used synthetic pyrethrin synergist and there are no reports available on toxic effects on humans resulting from the exposure to it. Piperonyl butoxide is not an insecticide itself but a cytochrome P450 inhibitor which allows pyrethroids such as permethrin to be much more active (10x). Inhibition of the detoxification pathway allows higher unchanged systemic concentrations of the active insecticide to remain within the target animal for a longer period. This is here now.

**Long-lasting insecticide treated nets**

Mass produced long lasting insecticide treated nets (LLINs) are replacing older style bed nets. Olyset net was the first LLIN which became commercially available. Sumitomo’s Olyset® technology incorporates permethrine insecticide directly into polyethylene filaments which can be woven into sturdy bed nets to provide long-lasting protection from night-time biting mosquitoes. Olyset Plus, which received WHO approval in July 2012, retains the controlled-release technology and durability, and contains 2% permethrin and 1% of the synergist piperonyl butoxide (PBO). The fibres have been designed to release the two ingredients at a constant ratio of 2:1. The ‘bleed rate’ at which permethrin and PBO migrate from the internal reservoir in the fibres to the surface of the net has been adjusted in order to make the net active again within 1-2 days of washing. For this work, Sumitomo Chemical became the co-winner of the 2012 ‘Application of Core Competence’ category – Global Business Coalition Health Award. A major production plant has been set up in Tanzania.

Fine-mesh gauze can be applied to windows and ventilation shafts. One good argument for using a mosquito net is the fact that it also protects from nuisance insects such as *Culex* mosquitoes and bedbugs. In regions where there are few *Culex*, people are not so ready to use a net: after all they cannot see or hear any mosquitoes (anopheline mosquitoes fly with little noise).

Insecticides based on pyrethrum can be dispersed by means of spraying (spray gun), evaporation (heated electric plate) or burning (mosquito coil, e.g. with esbiotrin). Insecticides can also be applied to the walls or to the curtains by the windows.

There are also various insect repellents. DEET (N,N-diethyl-m-toluamide, now called N,N-diethyl-3-methylbenzamide) is moderately active and can be applied as an alcoholic solution to the skin. This
produces a sticky effect when the alcohol evaporates. The effectiveness is only moderate. DEET is absorbed through the skin and is eliminated quickly via the urine. There is no accumulation in the body. The higher the concentration, the longer the duration of action: DEET 20-30% gives 4-6 hours protection, DEET 50% offers 8 hours protection. Concentration higher than 50% don’t give significant longer protection.

Alternative repellents are (p)icaridine (Care-Plus® Repel-it; Parazeet®) and IR3535 (Cinq sur Cinq®, Moustidose®).

**Intermittent preventive treatment (IPT) and seasonal malaria chemoprophylaxis (SMC)**

In highly endemic countries (sub-Saharan Africa), several “preventive” strategies have been promoted and adopted for special risk groups or for some periods of higher transmission. They consist of administering some drugs with antimalarial activity at regular intervals to a group of population with no previous diagnostic testing for malaria. The main aim is to control the malaria morbidity and important reductions of clinical and severe malaria or malaria-related complications (on fetus/newborns for example) have been repeatedly demonstrated.

At this moment, IPT use is recommended by WHO

- in pregnancy (ITPp) as part of antenatal care: sulfadoxine-pyrimethamine (SP) starting from the second trimester with at least three administrations at one-month intervals minimum
- in infants (< 12 years) during the immunization program: SP (where still effective) at the second and third rounds of vaccination against tetanus/diphtheria/pertussis and at vaccination against measles
- in children (< 6 years) in the sub-Sahel region during the rainy season: SP + amodiaquine once a month during each transmission season (strategy called SMC)

On an important prospective note, ACTs are also increasingly explored as IPT in various populations for preventive purposes. ITP with ACT is currently investigated in pregnant women, infants, children < 6 years, school-age children, whole population where malaria is about to be eliminated. This field and the related WHO recommendations are expected to evolve deeply in the coming years

**Chemoprophylaxis for travelers**

Chemoprophylaxis is in the first instance intended as prevention of *P. falciparum* malaria. No single
drug which is taken preventively is 100% active against sporozoites and no single drug prevents the formation of liver forms (except primaquine). While taking prevention no vivax or ovale malaria will occur but after they have been discontinued an attack with these plasmodia is possible in the following months or years.

In view of the extensive resistance of \textit{P. falciparum}, at present no 100% satisfactory protection against this latter parasite is possible. Advice as to whether or not to take medication and which kind of drug to take, will depend on the region and differ from person to person (short journeys, resident, local population, pregnancy, young children, allergy, chronic diseases, use of other drugs and so on). Recommendations vary from country to country and evolve in time.

- In regions with only \textit{P. vivax} and/or sensitive \textit{P. falciparum} (WHO type A) chloroquine 300 mg/week will suffice.
- In zone C with resistant/multidrug resistant \textit{P. falciparum}, 3 different regimens are currently recommended:
  - Atovaquone/proguanil 250/100 mg 1 tablet per day beginning 1 day before departure until 7 days after return
  - Doxycycline 100 mg/day during the stay and up to 4 weeks after return
  - Mefloquine 250 mg 1 tablet per week, to start two-three weeks before departure, and to continue up to 4 weeks after return

The decision should be individualized, since it depends on several aspects (side effect profile, type of trip, budget). Given the lower cost of generic drugs of atovaquone/proguanil and its good tolerance, atovaquone/proguanil is often chosen as the prophylactic treatment, especially for shorter journeys.

Doxycycline is an alternative in case of atovaquone/proguanil intolerance. Prolonged ingestion of doxycycline can lead to phototoxicity, including photo-onycholysis. Sunscreens do not block ultraviolet A well enough to prevent phototoxic reactions to doxycycline.

Today, the use of mefloquine as preventive treatment has decreased. The plasma half-life of mefloquine is 2 to 3 weeks. Ingestion of 1 tablet per week produces stable blood levels after 7 weeks. Traditionally it is said that mefloquine prophylaxis should be started before departure. This guideline is based on the consideration that intolerance to the drug can be monitored in this way. It is safe to begin the medication 15 days before departure so that 3 tablets are taken before leaving. In this way 75% of the side effects can be detected. At the prophylactic dosage (adults one 250 mg tablet per week) side effects occur in 2 to 3% of people, which require that the prophylaxis be discontinued. Rarely (1 in 12,000 to 15,000) preventive dosages may trigger epilepsy or psychosis may occur.
Epilepsy and arrhythmias (including the use of beta-blockers, calcium antagonists and digitalis) are contra-indications for the use of this product. Latest data indicate that it is proven safe during pregnancy. There are sufficient data that it is safe if taken for longer periods. The first case of mefloquine resistance was described in Thailand in 1982. There is already mefloquine resistance on a small scale in many countries, but this can be significant locally: e.g. the cure rate in East Thailand was only 41% in 1993. *P. falciparum* malaria can thus sometimes occur in spite of correct prophylactic use of mefloquine. Mefloquine does not kill sporozoites and liver parasites (therefore *P. vivax* and *P. ovale* malaria are still possible after leaving an endemic zone and after discontinuing mefloquine chemoprophylaxis).

For longer stays we recommend after a period of adequate chemoprophylaxis (a few weeks) at arrival; to travel with stand-by emergency treatment (SBET) of quality, to use in case of malaria, either breakthrough under chemoprophylaxis or attack occurring later. It is of utmost importance to remain alert in case of fever even after several years of tropical stay. Malaria is always possible, even in regions of lower transmission and malaria should be investigated appropriately and treated accordingly. Emergency treatment for travellers in 2016 includes Malarone®, Riamet® or Eurartesim®.

The local population should not take chronic chemoprophylaxis and most people develop semi-immunity. There are however some high-risk groups: e.g. pregnancy, children less than 5 years and HIV patients. During pregnancy particularly in the second and third trimesters and also immediately post-partum, the immunological resistance to malaria falls. Intermittent preventive therapy in pregnancy ("IPTp") protects against maternal anaemia and low birth weight, and its use in areas in medium to high transmission is recommended by WHO (in most African programs Fansidar is used). The efficacy of IPTp is reduced in HIV-positive women.

**Vaccination**

Research into a malaria vaccine is based on a number of possibilities. An immune response can be triggered against sporozoites and liver forms (pre-erythrocytic vaccines), erythrocytic forms (blood-stage vaccines) and/or gametocytes (transmission blocking vaccines). However the immune response does not necessarily have a protective effect. A 100% effective malaria vaccine is not likely to be developed in the foreseeable future but a vaccine which leads to partial protection is being evaluated in different fields.
**RTS,S/AS01**

In the early 1980s antibodies against sporozoites were used to identify the main antigen, circumsporozoite protein (CSP). The CSP is expressed on the surface of the parasite during the infective sporozoite stage.

In 1996 the first favourable results became known. A randomized and controlled study in the Gambia on 306 volunteers showed RTS,S/AS01 to provide significant protection against natural *P. falciparum* infection. The RTS,S/AS01 is a recombinant vaccine against the pre-erythrocytic stage of the parasite in which regions of *P falciparum* CSP are fused to hepatitis B surface antigen. It was developed by a public-private partnership with support from the Bill and Melinda Gates Foundation. The results of the large phase III trial that enrolled 15,459 infants was carried out at 11 clinical trial centers in seven countries (Burkina Faso, Gabon, Malawi, Mozambique, Ghana, Tanzania, Kenya) were published in 2012. In this trial 3 vaccines were given with a 1-month interval and some received a booster 20 months after the first vaccine to assess if higher immunity is maintained with a booster vaccine. Initial results demonstrated a vaccine efficacy of about 31% for both clinical and severe malaria in African children and a 26% vaccine efficacy against severe malaria. However, a follow-up study over 7 years showed that these results were offset by rebound in later years in areas with high exposure to malaria parasites. In year five to seven after vaccination, the vaccinated group even had a higher risk of febrile convulsions than the control group with a possible higher risk for cerebral malaria and meningitis in areas with high exposure. Nevertheless, pilot implementation studies are currently being initiated in Kenya, Malawi and Ghana and will learn whether large-scale use of the RTS,S/AS01 vaccine may enter future malaria preventive programs.

**PfSPZ**

PfSPZ is a newly developed vaccine, eliciting an immune response against *Plasmodium falciparum*. It is made of non-replicating irradiated whole sporozoites (SPZ), the parasite stage that infected mosquitoes inject during a bite. The vaccine is unique in using whole parasites as its ingredient. In healthy volunteers a strong protection was noted in lab studies with development of CD8+ T-cells producing IFNγ. These T cells play a key role in the immune response to fight malaria in the liver. The difficulty with this vaccine however is that PfSPZ must be injected intravenously, that poses challenges for mass vaccination campaigns. On top of this, it must be stored in liquid nitrogen at -195 °C or colder. Sanaria, the developing company, is developing a robot that can dissect salivary glands of mosquitoes. This step should make preparation and further development of the vaccine faster and easier.
A pilot trial that will enrol 2,100 people aged 2-50 years on the west African island of Bioko is being planned. If the first results are promising, the plan is to vaccinate another 10,000 people and ultimately all 280,000 habitants of the island. PfSPZ’s efficacy in the field will inevitably be lower than in lab studies because people might have weaker responses to the vaccine due to pre-existing exposure to malaria or local strains of the malaria parasite might differ from the one used in the vaccine. But combined with conventional measures such as indoor insecticide spraying and insecticide treated bed nets, there is the hope to be able to completely eradicate malaria on the island.

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Malaria - Treatment

General

Most people are not very interested in the history of a particular medicine. Quinine, however, is rather different and occupies a special place. For 300 years this was the only specific treatment for malaria. The story of its discovery, the important part which quinine has played in the colonization of the tropics, its role in both World Wars and during the Vietnam war, and the present come-back of this product all make it unique. At present quinine and related products are used in the treatment of \textit{P. falciparum} malaria, as an antiarrhythmic, as a muscle relaxant and as a flavouring (Schweppes!)\textsuperscript{[1]}. There are also some minor applications such as the treatment of babesiosis. Quinine is obtained from the bark of Cinchona trees.

In 1934 resoquine was discovered by the German H. Andersag. Only after the allies took North Africa there was renewed interest in the product. It was renamed chloroquine. Preparation in the laboratory was also economically viable. It quickly became the first choice agent and quinine was pushed into the background. In 1950 in Brazil, Mario Pinotti introduced the strategy of adding chloroquine to cooking salt (as was also done with iodine).

The synthetic preparation of primaquine was perfected after the war. The British war programme led to the development of proguanil, which itself served as a model for the development of pyrimethamine. Pyrimethamine in combination with sulphadoxine was introduced in 1970 under the name Fansidar\textsuperscript{®}. After World War II it was hoped that malaria would be definitively eradicated. The
use of chloroquine and the world-wide campaign to eradicate malaria by the World Health Organization, led initially to a considerable reduction in malaria infections all over the world. After the anti-malaria campaign vanished due to various circumstances, the resistance of Anopheles to various insecticides and the development of chloroquine-resistant and multi-resistant *P. falciparum*, malaria once more became one of the major problems.

Whereas World War II led to the discovery of some new anti-malaria agents, the Vietnam war stimulated a huge programme for the discovery of new drugs. The Walter Reed Army Institute of Research of the United States army investigated thousands of constituents. This research resulted in mefloquine (Lariam®) and halofantrine (Halfan®). Research in China produced artemisinin, pyronaridine and benflumetol.

**Treatment overview**

Broadly speaking, anti-malaria drugs can be divided into four major classes

- Blood schizonticides
- Antifolates
- Antimitochondrials
- Redox process-based agents

**Blood schizonticides**

When the malaria parasite leaves the liver and penetrates an erythrocyte, it can begin a haemoglobin diet. Chloroquine, quinine, mefloquine and halofantrine interfere with the detoxification of haemin in the digestive vacuole of the parasite, so that haemin can generate free radicals and parasitic membrane damage follows. It is therefore logical that the drugs are not active against the parasitic stages which precede the blood forms (sporozoites, liver forms) and which do not consume haemoglobin.

**Antifolates**

Folic acid is an important metabolic factor. Humans obtain this vitamin from the food they eat. The malaria parasite must produce it for itself. Para-aminobenzoic acid (PABA) is used at an early stage of the biosynthesis of folic acid by the enzyme dihydropteroate synthetase. This step is inhibited by structural analogues of PABA, such as sulphonamides and sulphones, e.g. sulphanilamide, sulphadoxine and dapsone.

The next synthesis step is catalysed by dihydrofolate reductase. This step is prevented by pyrimethamine, trimethoprim and cycloguanil (prodrug = proguanil), to such an extent that tetrahydrofolate – the end product – is not formed. The combination of these two sequential inhibitors forms the basis of Fansidar® (similar to cotrimoxazole). Resistance to both antifolates easily develops. A specific point mutation in each gene (dhps and dhfr) is sufficient.

**Antimitochondrial products**

Although artemisinin derivatives and 8-aminoquinolines (primaquine and tafenoquine) cause mitochondrial swelling, this organelle is not their chief target. Some antibiotics such as tetracycline and clindamycin prevent protein synthesis by mitochondrial ribosomes (these are similar to the ribosomes found in bacteria). They are slow-acting.
Atovaquone is a naphthoquinone which specifically destroys the electron transport chains of Apicomplexa. The molecule is similar to ubiquinone (coenzyme Q) which plays a role in the energy transfer between cytochrome B and C1. The enzymes of *Plasmodium falciparum* are 1000 times more sensitive to atovaquone than the corresponding enzymes in humans. Resistance can easily develop if used in monotherapy.

**Redox reactions**

Primaquine and tafenoquine exercise their action via redox-active quinone metabolites. They are selectively toxic for the pre-erythrocytic stages and are the only medicaments which kill hypnozoites. Tafenoquine has in addition a pronounced blood schizonticidal action.

**Current treatment of malaria**

A summary of the WHO recommendations in 2020 is provided first in these notes for clarity. For detailed dosages and special groups, see additional information in “Guidelines for the treatment of malaria: WHO; third edition, 2015.

All drugs used currently or in the recent past are described in detail below the summary.

- Complicated malaria (whatever the species, and also in all risk groups)
  
  **First choice:** Artesunate IV (2.4 mg/kg in adults and children > 20 kg; 3 mg/kg in children < 20 kg)

  **Second choice** (only if artesunate not available): quinine IV (see dosage below)

- Uncomplicated malaria (whatever the species)
  
  Artemisinin-based combination treatment (ACT); five ACTs are currently accepted; all are in fixed-dose combination (FDC) nowadays and consist of 3-day regimen:

  - Artemether-lumefantrine
  - Artesunate-amodiaquine
  - Artesunate-mefloquine
  - Artenimol (dihydroartemisinin)-piperaquine
  - Artesunate + sulfadoxine-pyrimethamine (SP)
NB1: In low-endemic countries, a single dose of primaquine (0.25 mg/kg) should be added at the end of the ACT to decrease transmission (no need of G6PD determination)

NB2: Chloroquine (see dosage below) is a good alternative for uncomplicated *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* in areas where no resistance is reported

NB3: Primaquine (30 mg/day for 14 days) should be administered in case of *P. vivax* and *P. ovale* infections, after determination of the G6PD activity (alternative regimens available in case of low activity)

**Anti-malaria drugs**

**Qinghaosu and Artemisinin derivatives**
Artemisia annua in Vietnam. This plant is harvested in order to extract artemisinin from the leaves. Copyright Charles Lugt (with special thanks to prof Kager).

Artemisinin and its derivatives have become essential components of antimalarial treatment. ACTs are now recommended by WHO as the first-line treatment for all falciparum malaria in malaria endemic countries. These plant-derived peroxides are unique among antimalarial drugs in killing the young intra-erythrocytic malaria parasites, thereby preventing the more pathogenic mature stages. Huang hua hao or qinghaosu (“essence of qinghao”) originates from a Chinese plant, Artemisia annua (sweet wormwood). The antimalarial properties of the traditional Chinese medicine qinghaosu were discovered and developed by Chinese scientists in 1971 (secret “project 523”). This research effort was prompted by the requests of Ho Chi Minh to Zhou En Lai for antimalarial drugs for the Vietnamese troops (cfr the efforts of the American forces to develop halofantrin and
mefloquin).

Artemisinin has the derivatives artesunate (the hemisuccinate; -CO(CH2)2COOH), arteether (the ethyl ether; -OCH2CH3), artemether (the methyl ether; -OCH3) and the reduced substance artemimol, syn. for dihydroartemisinin. Their plasma half-life is very short: 1 hour, both in healthy volunteers and in patients with active malaria.

Artemisinins are not active upon liver stages, but upon both the immature sexual and the all asexual blood stages. Their broad stage specificity (as opposed to quinine) has several therapeutic consequences. Killing young circulating ring-shaped trophozoites results in a more rapid reduction in parasitaemia as compared to other antimalarials and reduces the number of parasites that mature and sequester in the post-capillary venules. Quinine does not stop sequestration since it acts on the mature parasite stages, which have already adhered to the vascular endothelium. Since artemisinin reduces the number of gametocyte carriers, it helps to prevent malaria transmission, although artemisinin does not kill mature gametocytes of \( P. falciparum \). In low-transmission areas, where symptomatic infection constitutes the main source of transmission, ACTs reduce gametocyte carrier rate, and if widely employed is expected to reduce the incidence of malaria. Artemether, artesunate and dihydroartemisinin reduce the number of parasites by a factor of approximately 10,000 for each asexual cycle. After two cycles (3-day treatment) there is a \( 10^8 \)-fold reduction of the parasitaemia. The longer acting partner drug will then eliminate the remaining low numbers of parasites.

The medication is best avoided during the first trimester of pregnancy, if a good alternative is available (quinine + clindamycine). Recent large studies (PREGACT) have demonstrated the safety of ACT administered during the second and third trimesters on pregnancy and infant outcome. In observational studies of pregnant women treated with artemisinin derivatives during the first trimester, no differences were noticed in the risk of miscarriage, stillbirth or congenital anomalies when compared to quinine treatment. Although data are limited, the use of ACTs is probably safe throughout gestation, especially if alternatives are not available.

Artemether (Paluther®, Arteminth®, Cotexcin®, Artenam®) is an oil-soluble derivative that can be used for IM administration.

Artesunate (Artenam®, Artesunate®, Arsumax®, Artemax®, Arinate®, Plasmotrim®) is the fastest-acting artemisinin derivative. It can be administered parenterally (IV, IM), rectally or orally. For IV use the dose is 2.4 mg/kg as start dose. This dose is repeated at least after 12 hours and 24 hours. The side-effects are mild and are difficult to distinguish from the effects of malaria itself. However, delayed onset haemolytic anaemia has been observed in about 20% of travellers who receive
artesunate after about 2 weeks. This post-artesunate delayed haemolysis is also described in endemic countries. Haemoglobin monitoring 1 and 2 weeks after artesunate administration is strongly recommended for this reason, particularly after an episode of severe malaria.

There is now strong pharmacological and clinical evidence that artesunate is superior over quinine for treating severe malaria (35% reduction of fatalities in Asian adults and 22% reduction of fatalities in African children). If patients with severe malaria cannot be treated orally and transport to a hospital for IV therapy will take more than 6 hours, a single inexpensive artesunate suppository at the time of referral substantially reduces the risk of death or permanent disability. A single dose of artesunate, given rectally (by e.g. parent), can provide parasiticidal blood concentrations within 10-20 min and can already halve parasitaemia numbers within 6-12 h.

Artenimol (more commonly named dihydroartemisinin) is obtained by reduction (hydrogen addition) of artemisinin. Together with piperaquine it is available as a fixed drug combination known as Eurartesim®. Artenimol has a short half-life, as opposed to piperaquine which has a long half-life.

After a decade of use in monotherapy in Southeast Asian countries, it has become clear that monotherapy would quickly lead to resistance to artemisinin derivatives (5-10% recrudescence after 7 days of monotherapy). Since 2005, to protect this “last-line” drug, WHO has strongly recommended to systematically combine artemisinin with another, partner drug with a longer half-life to treat all falciparum malaria in endemic countries. “Accepted” partner drugs are amodiaquine, pyrimethamine/sulphadoxine, lumefantrine, piperaquine or mefloquine (see other drugs). New ACT compound are emerging such as pr

Artemisinins also have some activity against other parasites, for example they kill the young stages of trematodes such as schistosomes and Fasciola. They are studied also in animal models of clonorchiasis.

**Lumefantrine or Benflumetol**

Lumefantrine (= benflumetol) was registered in China in 1987 for the treatment of *P. falciparum* malaria. The half-life in the blood is approximately 4 days. The product is not active on the liver stages or gametocytes. Lumefantrine, like chloroquine, probably destroys heme polymerization (a detoxifying pathway for the parasite). It is synergistic with artemether. The combination artemether-lumefantrine is known as co-artemether (AL; Riamet®, Coartem®: artemether 20 mg/lumefantrine 120 mg, adult dose 2×4 tablets/d for 3 days). The combination artemether-lumefantrin is probably the most used ACT worldwide.
The possibility of drug-interaction and QTc-prolongation needs to be studied further, especially if this product would be used as stand-by medication in travellers to the tropics who also might take certain quinolones, azoles, macrolides or prokinetics (domperidone).

Absorption in the intestine is highly variable from person to person and is greatly increased (up to 16-fold) by fatty food. Since people who are ill generally do not eat much, this has important consequences. Early in the treatment very little lumefantrine is absorbed. In combinations, such as Co-artem, the artemether is responsible for the initial important reduction in the number of parasites and the low residual numbers of parasites is then cleared up by lumefantrine.

In HIV-infected children, lopinavir-ritonavir-based ART (Kaletra) was associated with a decreased incidence of recurrent malaria (reinfection) as compared to an NNRTI-based regimen, largely because of an interactions that increases drug levels of lumefantrine.

**Piperaquine**

Piperaquine is a Chinese synthetic drug belonging to the bisquinolines. Half-life of piperaquine is 9 days. Piperaquine is a highly lipid-soluble drug. The combination dihydroartemisinin (artenimol) 40 mg with piperaquine 320 mg per tablet (Artekin®, Eurartesim®, Duo-cotecxin®, adult dose: 1×4 tablets/day for 3 days) is increasingly used in first-line in many endemic countries.

In 2006 Papua New Guinea became the first country to implement dihydroartemisinin-piperaquine treatment for *P. falciparum* and *P. vivax* infection in pregnant women during the second and third trimesters as well as its first-line therapy for any case of malaria. Because of the slow elimination of piperaquine, this treatment provides up to 6 weeks posttreatment prophylaxis against new infections and relapsing *P. vivax* infection (better than all other ACTs). It is recommended in travel medicine to check first an ECG to exclude an underlying QTc prolongation in people with serious liver, kidney or heart diseases or in people taking other QTc prolonging medication (macrolides, fluoroquinolone, domperidone, ...). It is contra-indicated if > 500 msec and to be used with caution if QTc > 450 msec.

**Amodiaquine**

Amodiaquine is closely related to chloroquine. Long-term use causes grey skin pigmentation in white people. Sometimes there are severe side effects (agranulocytosis in approximately 1/2000, liver toxicity in approximately 1/15,000). Amodiaquine (Camoquine®, Flavoquine®, Malarid®) had been rarely used in monotherapy. There is therefore less resistance to amodiaquine than to chloroquine.
Since the product is eliminated slowly, a single dose of 600 mg was (and is) sufficient.

Amodiaquine is nowadays the partner drug of artesunate in one of the 5 recommended ACTs. This therapy exists now in fixed-drug combination (Coarsucam®, ASAQ: 100 mg artesunate/270 mg amodiaquine, adult dose: 1×2 tablets/d on 3 consecutive days) and because of its low price, has become the first-line ACT for P. falciparum in many African countries.

**Mefloquine**

Mefloquine (Lariam®) is a long-acting product. After 2 to 3 weeks half of the dose is still present in the body. Mefloquine has a rather slow onset of action. For curative use, mefloquine is always combined with other antimalarials, and its use in monotherapy for treatment is now strongly discouraged (major side effects, while effective alternatives exist). The combination mefloquine + pyrimethamine + sulphadoxine is known as Fansimef®. Now, mefloquine is used with artesunate in a fixed-drug combination and is one of the first-line therapies of Pf malaria in many countries: artesunate 100 mg/mefloquine 220 mg (ASMQ), 1×2 tables/d for 3 consecutive days (adult dose).

Mefloquine plays an important (although decreasing) role in prophylaxis: cfr. infra.

**Quinine**

Quinine has long been a first line anti-malarial drug and was for a long time one of the only parenteral treatment options. More recent studies however, showed clinical benefit of parenteral Artesunate and oral artemisinin combination treatment over quinine, together with less side effects. Quinine is still a powerful product, which acts upon the schizonts of the parasites in the blood (it is a schizonticide). It thus acts chiefly in the second half of the maturation cycle: on the parasites which are sequestered in the small blood vessels (not on the young ring forms in the peripheral circulation). Quinine also possesses gametocytocidal activity against *P. vivax, P. malariae* and *P. ovale* (but not against gametocytes of *P. falciparum*). As for chloroquine, quinine causes an inhibition of hemozoin biocrystallization in the heme detoxification pathway, which facilitates the aggregation of cytotoxic heme. Free cytotoxic heme accumulates in the parasites causing their deaths.

Quinine sulphate is administered orally. It is absorbed well in the intestines. Quinine bihydrochloride is injected, preferably by slow IV (infusion with glucose because of the risk of hypoglycaemia). IM injections may lead to sterile abscesses but can be used where necessary if there are no alternatives available. For IM injection, it is best to use a diluted solution (60 to 100 mg/ml) instead of the
concentrated solution (300 mg/ml). Quinine administered via IM injection is absorbed well even in severe malaria. Treatment with quinine is unpleasant (bitter taste, cinchonism) and poor compliance after the acute phase is common.

**Treatment regimens**

The basic regimen is 10 mg salt/kg, every 8 hours, orally or slow IV. Currently, a loading dose of 20 mg/kg IV over 4 to 8 hours is universally recommended for the first administration (followed by 10 mg/kg every 8 hours). This should be continued for at least 4 days, preferably 7 to 10 days (if used in monotherapy). This is an unpleasant treatment. Because there is still a risk of relapse if quinine is used in monotherapy even for > 7 days, another product is generally combined with it, e.g. tetracycline or clindamycin. This allows also to shorten the quinine administration to 4-5 days. Sometimes treatment with Fansidar® is given after a few days, which shortens the treatment period, but only in regions where this drug is still sufficiently effective. If a patient vomits within an hour after swallowing the medication, the whole dose should be repeated. If vomiting occurs longer than one hour after ingestion, no new dose is necessary. In case of repeated vomiting IV administration is required.

**Side effects of quinine**

Quinine is a substance with highly irritating properties (also for the gastric mucosa: nausea is not uncommon). Capsules are therefore best taken after a meal. Quinine increases the secretion of insulin from the pancreas, increasing the risk of hypoglycaemia. Quinine allergy is not common. What is common is a range of side effects such as tinnitus, temporary deafness for high frequencies, headache, nausea and palpitations. These toxic phenomena are known as cinchonism: quinine was first isolated from the bark of the cinchona tree. This reduces the patient’s compliance.

Quinine increases irritability of the pregnant uterus. In case of need one must not hesitate to use quinine in a pregnant woman with malaria (malaria itself can lead to abortion, preterm labour or death in utero). To prevent an impending premature labour, a tocolytic agent can be given such as the beta 2-mimetic ritodrine, fenoterol or salbutamol. The calcium antagonist nifedipine is as effective a tocolyticum as the beta-mimetics. Prolongation of the PR, QRS and QT intervals may occur during the use of quinine (as with quinidine). If the patient has atrial fibrillation, conversion to sinus rhythm may occur with possibly arterial embolic complications. Atrial fibrillation which has already been present for more than 48 hours is a contra-indication for quinine. Congenital long QT syndrome and Brugada syndrome are equally formal contra-indications for using quinine. ECG monitoring to detect QTc-prolongation is recommended during quinine therapy, especially in case of kidney failure.
Overdose of quinine may lead to very severe situations such as deafness, delirium, bradycardia, hypotension, respiratory arrest or death (lethal dose approximately 8 grams). Overdose may also lead to blindness via a direct toxic effect on the retina and possibly also due to spasms of the retinal blood vessels and subsequent retinal ischemia.

**Quinine and Gin Tonic**

Unlike the majority of other bitter products which occur naturally, the bitter taste of quinine is short-acting with no annoying after-taste. It is therefore used as a flavouring to produce tonic water. The British colonialists in India often drank gin and tonic. The present-day tonic water contains approximately 15 mg per litre, however, only enough to give a bitter taste. Copious drinking of gin and tonic in order to prevent malaria, is thus only an excuse for drinking gin.

**Why is quinine resistance still rare?**

The product has been used for more than 360 years. This is in stark contrast to the resistance to other malaria drugs or antibiotic resistance in bacteria where the “useful life” of a product is measured in years or a few decades. The concept of a standard dose was only developed in the twentieth century. Earlier the duration of treatment and the dosage were left to the discretion of the doctor. This together with the fact that the concentrations of alkaloids varied greatly from plant to plant and that quinine was never pure, meant that malaria was treated with a therapy which must have produced the most varied blood levels. Yet no widespread quinine resistance has been reported. The answer to the question why there is virtually no quinine resistance, could be very important. Is the target molecule of quinine so special that mutation is not possible? It would then be very helpful to know this target. It could also be that there is quinine resistance, but that it was not, and has not been recognized. However, this is doubtful. Is it that the present recommended dose is much higher than that which was formerly necessary? Is it the fact that “quinine” is actually a mixture of various active products, which prevents resistance developing? Resistance to combined therapy requires multiple, simultaneous mutations which is less readily achieved than that to single products. It is possible that quinine has not previously been used at levels which create sufficient evolutionary pressure. The majority of malaria cases in Europe and America were *P. vivax* infections. Even in British India, *P. vivax* represented the lion’s share of infections. In *P. falciparum* endemic regions, only a few fortunate people were able to take quinine and then only when they had to (because of unpleasant side effects). Few used quinine as a prophylactic agent (especially among the indigenous population). What is more, quinine has a short half-life, so that the parasite was only exposed to subtherapeutic concentrations for a short time. Probably its
limited use is the reason for the absence of resistance, but if used on a larger scale, quinine resistance may yet become a reality in years to come.

**Chloroquine**

Despite the presence of this resistance, chloroquine still has a place in treatment. It is still active against non-falciparum plasmodium species almost everywhere and could theoretically still be used against chloroquine-sensitive *P. falciparum* in very limited areas: Central America and the Caribbean. Elsewhere, chloroquine is not recommended any more against *P. falciparum* even in immune patients, who do not usually appear very ill.

The trophozoite in the red blood cell breaks down haemoglobin using lysosomal enzymes. In this digestive process ferriprotoporphyrin IX (haemin) is formed, which is toxic to the parasite and is usually polymerized to non-toxic malaria pigment. Chloroquine binds to ferriprotoporphyrin IX and prevents detoxification.

Since the liver parasite do not feed on haemoglobin this drug is not active at the pre-erythrocytic stages of *Plasmodium sp*.

Chloroquine is available in tablet form as chloroquine sulphate (Nivaquine®) and as chloroquine diphosphate (Resochine®). Hydroxychloroquine sulphate (Plaquinil®) is different and is used in e.g. rheumatoid arthritis, lupus erythematosus and Q-fever. The injectable form is chloroquine dihydrochloride. Nivaquine® tablets contain 100 mg chloroquine, but availability of this drug has decreased over the last years.

Chloroquine is a powerful schizonticide. It has strong affinity for various tissues and organs. It is fast-acting and remains in the blood for many days. A brief treatment (3 days) is therefore possible.

Chloroquine may be given orally, SC, IM or SLOW IV (infusion). Never inject an ampoule of chloroquine IV rapidly as a bolus or rapid infusion (fatal arrhythmia). The injections are not painful.

There are several different treatment regimens. Most of the time it is given orally, 25 mg/kg spread over three days. Parenteral administration should be discontinued as soon as oral administration is possible.

Chloroquine is cheap and not very toxic in normal use.
- Some people are allergic (pruritus, rash) or suffer nausea.
- People with psoriasis are more at risk of side effects.
- A reversible precipitation of chloroquine in the cornea may occur, resulting in small opacities. This may result in seeing haloes around objects, blurred vision or photophobia. This form of keratopathy may become manifest quite rapidly (a few weeks after beginning treatment). After discontinuing the medication it is completely reversible.
- Chloroquine accumulates in melanin-containing tissues. Chronic use may lead to abnormalities of the choroid and retina (chorioretinitis). This toxic retinopathy is not reversible. The abnormalities are always bilateral and symmetrical. Often there is maculopathy (bull’s eye lesion) with central and paracentral scotomata, but constriction of the peripheral field of vision may also occur. The total cumulative dose before such problems occur is generally 100 gram chloroquine or more.
- Chloroquine has a narrow safety margin (just 30 mg/kg may be fatal). In case of overdosage myocardial depression, hypotension and severe arrhythmias may occur. ST-segment abnormalities and T-wave inversion occur. Broadening of the QRS complex (>0.12″) and ventricular arrhythmias have a poor prognosis. The patient may become comatose, vomit and aspirate stomach contents. In acute intoxication diazepam is given (Valium® 1 mg/kg) and adrenalin (= epinephrine) or dopamine if these are available.

**Pyrimethamine + / – Sulphonamides**

Fansidar® is a combination product of pyrimethamine 25 mg and sulphadoxine 500 mg per tablet (Mekalfin® is another commercial name). The curative treatment for an adult is 3 tablets taken as a single dose. Sulphadoxine is a long-acting sulphonamide (t½ = 8 days) which in case of allergy may cause severe skin lesions (erythema multiforme and Stevens-Johnson syndrome). *Plasmodium falciparum* has rapidly developed resistance to this product in many parts of the world. It is not used any more as monotherapy but may be combined to artesunate (at least in regions where no resistance has been observed): Sulfamon®, Artesospe adult® (AS+SP) = artesunate 100 mg + sulphadoxine/pyrimethamine 500/25 mg: 1×2 tablets AS/d for 3 days + 3 tablets SP single dose. This combined treatment is available in co-blisters packs (this is not the same as coformulated tablets!). Fansidar is also widely used as intermittent preventive treatment for pregnant woman in Africa (either they present with blood parasite or not, once or twice during pregnancy) and still provide substantial benefit in preventing maternal and infant anaemia and low-weight birth (even in areas with increasing resistance). Though recent studies comparing dihydroartemisine-piperaquine (and other artemisinin-based combination therapies [ACTs]) vs pyrimethamine-sulphadoxine as intermittent preventive treatment during pregnancy (IPTp), showed that ACTs are usually superior in decreasing the malaria burden during pregnancy. Use of ACT in IPTp is however not yet a WHO recommendation, pending results on the long-term risk of developing resistance.
Halofantrine (Halfan®)

This is fast-acting, effective and has few but potentially lethal side effects. Given a series of casualties, it is no longer used and production has been abandoned. It has been replaced by a similar but non-toxic product: lumefantrin. Halofantrin was very dangerous in people with a long QT interval: reportedly lumefantrin does not present the same toxicity, but this deadly experience with halofantrin makes clinicians very cautious, ordering always an ECG before treatment whenever possible and almost always in high income settings.

Primaquine

Primaquine is an 8-aminoquinoline. It is inactive upon asexual blood forms. It does have an important though only partial causal prophylactic effect (on both *P. falciparum* and *P. vivax*) but only if it is taken 24-48 hours (max. 96 hours) after inoculation with sporozoites. It acts upon the exo-erythrocytic stages of the parasites (liver schizonts). The half-life is relatively short (4 hours). For causal prophylactic use a daily dose of 15-30 mg may be taken. These regimens are not very popular and there has been little experience of them. Chemoprophylaxis with primaquine can be stopped 3 days after leaving a malarious area.

In cases of *P. vivax* or *P. ovale* malaria, hypnozoites remain in the liver after therapy with ACT or chloroquine/quinine. These may be destroyed by primaquine. In the past, 15 mg base per day was used for 14 days [26 mg primaquine biphosphate = 15 mg primaquine base], but current medical opinion favours 30 mg per day for 2 weeks (increasing tolerance of some *P. vivax* strains). This drug is contra-indicated in pregnant women and in people with a significant deficiency of G6PD (glucose-6-phosphate dehydrogenase), an enzyme in the red blood cells (risk of haemolysis in patient and/or fetus).

Primaquine also acts on *P. falciparum* gametocytes. Therefore, in some circumstances (e.g. refugee camps) it may be given to reduce transmission (single dose of 45 mg). It is nowadays thoroughly investigated (in low dosage) as a potential strategy to decrease/suppress transmission in low-endemic areas contemplating elimination. Detection of underlying G6PD-deficiency is however a major hurdle for its use on a larger scale. Reliable point-of-care tests to detect G6PD deficiency would remediate this problem. Several low-endemic countries have already adopted the systematic administration of primaquine (0.25 mg/kg) at the end of the course of antimalarials/ACT administered to treat a clinical malaria episode. Mild methaemoglobininemia is often observed with primaquine but rarely with clinical consequences.
**Tafenoquine or Etaquine**

Etaquine or tafenoquine is a new 8-aminoquinoline, derived from primaquine. It has a half-life of two weeks, which is much longer than the half-life of primaquine. It may be taken orally and has low toxicity. It is active against *P. falciparum* and *P. vivax*. It is an effective schizonticide and is also active on the pre-erythrocytic stages, including the hypnozoites of *P. vivax*. Screening for G6PD deficiency is also required and this is always a limiting factor in low-resource settings. Tafenoquine has been approved in 2018 for the radical (relapse-preventing) treatment of *P. vivax* and *P. ovale* malaria (single dose of 300 mg just after the treatment of the clinical episode) and for malaria chemoprophylaxis (200 mg weekly). The experience of this new drug is not yet that large in clinical practice, but it is expected that replace primaquine soon, due to its much shorter/easier administration.

**Proguanil and Chlorproguanil**

Proguanil (Paludrine®) and chlorproguanil (Lapudrine®) are biguanides which are converted in the body to the active product cycloguanil.

The combination of chlorproguanil with dapsone is also known as Lapdap®. It is used as a cheap, short-half-life antifolate. It may be combined with artesunate (combination known as “CDA or Chlorproguanil-Dapsone-Artesunate”). In Malarone®, proguanil is combined with atovaquone and both drugs have a synergetic effect explaining its increased efficacy (despite the use of two drugs with moderate activity).

**Atovaquone**

Atovaquone (Wellvone®, Mepron®) is a lipophilic hydroxynaphthoquinone. Atovaquone is a powerful schizonticide for *P. falciparum* and *P. vivax*. On monotherapy recrudescence occurs very quickly. To avoid this problem, it is combined with proguanil (brand name of the atovaquone + proguanil combination = Malarone®). Atovaquone/proguanil is both used in curative and prophylactic regimen. It cannot be used in renal failure because the blood levels of proguanil/cycloguanil are much higher. Simultaneous use of atovaquone/proguanil and rifampicin is not recommended (blood levels 50% lower). Most recent data state that it’s probably safe in pregnancy, even during the first trimester. The curative dose is 4 tablets of atovaquone/proguanil 250/100 mg for 3 consecutive days.

The product is also being studied in toxoplasmosis, babesiosis, leishmaniasis, microsporidiosis and in
Pneumocystis jirovecii pneumonia. In the treatment of babesiosis it proved more active in some animal studies than the combination of clindamycin/quinine.

In general atovaquone/proguanil is very well tolerated. Nausea, diarrhoea and headache are the most frequent side-effects. Stevens Johnson syndrome has also been described. Resistance to atovaquone/proguanil has been rarely described even though a single mutation is enough to substantially decrease its activity. The limited use due to its high price might explain in part the lack of resistance.

**Miscellaneous products**

Tetracycline, minocycline and doxycycline are antibiotics which are active against malaria parasites but are very slow-acting. For this reason, they are never given as monotherapy, but in combination with quinine. They very much reduce the risk of relapse. Doxycycline has the advantage that it only needs to be administered once daily. Doxycycline is is sometimes used for malaria prophylaxis (cfr. infra).

Clindamycin (Dalacin®) is also active against plasmodia but is a second choice drug (risk of pseudomembranous colitis due to Clostridioides difficile). It is given together with quinine for Pf attack during pregnancy.

LAST UPDATED BY ADMIN ON DECEMBER 19TH, 2022

**Leishmaniasis**

**Summary**

- In humans obligate intracellular parasite with replication in macrophages
- Cutaneous form: chronic painless ulcers or nodules, amastigotes in smear
- Visceral form: chronic fever, hepatosplenomegaly, pancytopenia, persistent inflammatory state. Lethal if not treated
- Diagnosis of kala azar: amastigotes in bone marrow and other sites, serology, antigen detection
- Mucocutaneous: chronic destructive lesions in mouth/nose, frequent clinical diagnosis
- Transmission via about 30 species of sandflies
- Zoonotic transmission: animal reservoir (especially dogs and rodents)
• Anthroponotic transmission: human reservoir, e.g. Indian kala azar and in cutaneous *L. tropica*
• Treatment with antimony derivatives, amphotericin B, miltefosin, pentamidine. Combination treatment increasingly in use.

**General**

![Image of Leishmania braziliensis ulcer on the wrist and spread via the lymphatics. Lesions occurred after a visit to rural Bolivia. Copyright ITM](https://example.com/image.jpg)
Diffuse cutaneous leishmaniasis due to infection with Leishmania aethiopica. Copyright ITM

There are several species of *Leishmania* parasites and these can cause various clinical conditions. They can be responsible for chronic ulcers and skin nodules. Sometimes both skin and mucosae are affected (mucocutaneous leishmaniasis). When deep organs are affected, the condition is called visceral leishmaniasis. The *Leishmania* species that cause these various clinical conditions always have the same morphology under the microscope. However, there are differences in parasite DNA, proteins, enzymes and mode of development in the insect vector, etc. *Leishmania* parasites can in turn be infected with a RNA virus (the “leishmania virus”) though the significance of this is not yet known.

The classification, distribution and pathogenicity of the various *Leishmania* species is quite complicated. New data are regularly becoming available (for example, *L. tropica* was shown to be able to cause visceral leishmaniasis in rare cases). The whole taxonomy will probably change as more and more genetic information becomes available. A distinction is made between zymodemes (iso-enzyme patterns), schizodemes (kDNA analyses with restriction enzymes), serodemes (via reactions with monoclonal antibodies) and rapdemes (using PCR with random primers). Some 30 different *Leishmania* species have been described (10 in the Old World and 20 in the New World). Many of these can infect humans. The genus *Leishmania* is frequently subdivided into the subgenera *Leishmania* and *Viannia*. There are substantial geographical genetic variations. Hence in the dry western part of Peru *L. peruviana* causes the disease “uta”, an ulcerative form without mucocutaneous lesions. This organism contains less DNA in some of the chromosomes than the virulent *L. braziliensis*, the pathogen causing Espundia, a disease which occurs in the forests on the other side of the Andes in Eastern Peru. One of the differences is the number of copies of the leishmanolysin gene, which codes for an important surface antigen (gp63). This zinc protease has a role in adhesion to macrophages and survival in the phagolysosome. It is regarded as an important virulence factor. *L. braziliensis* contains more leishmanolysin genes than *L. peruviana*. The protein is being studied as, among other things, the basis for an experimental vaccine.

**Classification**

There is still no generally accepted internationally agreed definitive taxonomy. The following is for orientation:

<p>| Leishmania species |</p>
<table>
<thead>
<tr>
<th>New World</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. (Viannia) braziliensis</em></td>
<td>LCL, mucosal</td>
<td>zoonotic</td>
<td>Latin America</td>
</tr>
<tr>
<td><em>L. (Viannia) panamensis</em></td>
<td>LCL, mucosal</td>
<td>zoonotic</td>
<td>Northern South America and southern Central America</td>
</tr>
<tr>
<td><em>L. (Viannia) peruviana</em></td>
<td>LCL</td>
<td>zoonotic</td>
<td>Peru</td>
</tr>
<tr>
<td><em>L. (Viannia) guyanensis</em></td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td><em>L. (Viannia) lainsoni</em></td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td><em>L. (Viannia) columbiensis</em></td>
<td>LCL</td>
<td>zoonotic</td>
<td>Northern South America</td>
</tr>
<tr>
<td><em>L. (Leishmania) amazonensis</em></td>
<td>LCL, DCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td><em>L. (Leishmania) mexicana</em></td>
<td>LCL, DCL</td>
<td>zoonotic</td>
<td>Central America, Mexico</td>
</tr>
<tr>
<td><em>L. (Leishmania) pifanoi</em></td>
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<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td><em>L. (Leishmania) venezuelensis</em></td>
<td>LCL</td>
<td>zoonotic</td>
<td>Northern South America</td>
</tr>
<tr>
<td><em>L. (Leishmania) garnhami</em></td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
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<table>
<thead>
<tr>
<th>Old World</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. (Leishmania) aethiopica</em></td>
<td>LCL, DCL</td>
<td>zoonotic</td>
<td>Ethiopia, Kenya</td>
</tr>
<tr>
<td><em>L. (Leishmania) killicki</em></td>
<td>LCL</td>
<td>zoonotic</td>
<td>North Africa</td>
</tr>
<tr>
<td><em>L. (Leishmania) major</em></td>
<td>LCL</td>
<td>zoonotic</td>
<td>North and East Africa, Middle East, Central Asia</td>
</tr>
<tr>
<td><em>L. (Leishmania) tropica</em></td>
<td>LCL</td>
<td>anthropotic</td>
<td>North Africa, Middle East, Central Asia</td>
</tr>
<tr>
<td><em>L. (Leishmania) donovani</em></td>
<td>LCL, visceral</td>
<td>anthropotic</td>
<td>Central Asia, Africa</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Old and New World</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. (Leishmania) infantum</em></td>
<td>LCL, visceral</td>
<td>zoonotic</td>
<td>South Europe, North Africa, Central and South America</td>
</tr>
</tbody>
</table>

Leishmania species
Visceral leishmaniasis is mainly caused by the *Leishmania donovani* complex. There are several species in this complex:
1. *Leishmania donovani* (India, Pakistan, sub-Saharan Africa, East Africa)
2. *Leishmania infantum* (Mediterranean Basin, Middle East)
3. *Leishmania chagasi* (South America = *Leishmania infantum*)
4. *Leishmania archibaldi* (Africa) – of unclear importance

In the Old World skin lesions are mainly due to:

1. *L. tropica* (Mediterranean basin, Middle East). Frequently dry lesions
2. *L. major* (Middle East, sub-Saharan Africa). Frequently moist lesions
3. *L. aethiopica* (Ethiopia, Kenya). Sometimes also affects mucosa
4. *L. killicki* (North Africa) – of lesser importance

In addition, *L. infantum* and *L. donovani* (more exceptionally) can also cause skin lesions.

In (mainly South and Central) America skin lesions are caused by the *L. mexicana* and *L. braziliensis* complex. These complexes are subdivided into species:

1. *L. mexicana* complex: *L. mexicana*, *L. venezuelensis*, *L. amazonensis*
2. *L. braziliensis* complex: *L. braziliensis*, *L. panamensis*, *L. guyanensis*, *L. peruviana*

Mucosal lesions are common in infections with *L. braziliensis*. One should always keep in mind that the clinical lesions of leishmaniasis are a consequence of the parasite species on the one hand and of the immunological resistance and reaction of the patient on the other.

Infections occur very rarely with other *Leishmania* species: *L. (Viannia) naiff*, *L. (Viannia) shawi*.

**Distribution**
Map Leishmania infantum, L. aethiopica, L. tropica, L. major. Adapted from Colour Atlas
Map Leishmania mexicana and L.braziliensis. Adapted from Colour Atlas.

Map of the areas endemic for Leishmania chagasi, L. infantum, L. donovani, pathogens leading to kala azar. Adapted from Colour Atlas.
Mucocutaneous leishmaniasis occurs in Central and South America and occasionally in East Africa.

Visceral leishmaniasis occurs from western China to the Mediterranean Basin, East Africa and Central and South America. It is very rare in Africa south of the equator. The majority of cases occur in 6 countries: Bangladesh, Nepal, India, Ethiopia, Sudan and Brazil.

The cutaneous form is seen from India to the Mediterranean Basin, the northern half of the African continent and in Central and South America.

Leishmaniasis does not occur in Northern Europe, Canada, Uruguay, Chile, South Africa, Australia and Oceania. While Southeast Asia was thought to be leishmania free, an increasing number of visceral leishmaniasis cases have been reported from Thailand more recently.

For additional information and geographical risk in Europe; see www.leishrisk.net

**Vector**

The parasite is transmitted by the bite of infected female sandflies: *Phlebotomus* in the Old World and *Lutzomyia* in Central and South America. These genera, together with the blood-sucking genus *Sergentomyia* [little significance for man, as they suck blood from reptiles], belong to the Psychodidae family. Morphologically they very closely resemble each other. The name “sandfly” can be confusing as this name is sometimes used for other species as well. Sandflies are vectors of leishmaniasis, pappataci virus (an arbovirus) and *Bartonella* bacteria.
Sandfly. Lutzomyia and Phlebotomus species are vectors of leishmaniasis in the New, resp. Old World. Photo Cochabamba, Bolivia

Only some 10% of the approximately 600 known species of sandflies are vectors, and only 30 of these are important. A fly remains infected for life. In endemic areas, a minority of sandflies are infected usually below one per cent.

The female insects need blood in order to lay their eggs. Most species bite at night and at dusk. There are exceptions to this, such as *Lutzomyia wellcomei*, the main vector of *L. braziliensis*, which bites mainly during daytime. They can suck blood both from animals (cats, dogs, various rodents, cattle, birds and lizards, etc.) and man. They are small, soundlessly flying insects (approximately 2 mm in length). Because of these small dimensions they can get through standard mosquito nets. Impregnation with permethrine (cf. malaria) can help. Because of the very short mouthparts of the insects, they cannot bite through clothing. They are poor flyers. They will usually fly quite low and will remain in the vicinity of their breeding ground. They will also not fly when there is any wind. This knowledge can be exploited by having a fan or ventilator on at night in the bedroom to prevent
sandflies from flying. They require high humidity and temperature for breeding, although they can be observed in dry regions provided there are sites with a favourable local microclimate (crevices, termite mounds, caves, hollows and holes in tree roots, etc) where 15 to 80 tiny eggs can be laid. The larvae cannot survive drying out. They will feed on organic waste and then pupate. Sandflies reproduce optimally at 23-28°C and at a relative humidity of 70-100%. Temperatures below 10°C or above 40°C are unfavourable for their survival. Measures used to control adult sandflies include the use of insecticides for residual spraying of dwellings and animal shelters, space-spraying, insecticide-treated nets, impregnated dog-collars and personal protection through application of repellents/insecticides to skin or fabrics. Bednets will be most useful in areas with peridomestic vectors (e.g. *P. argentipes* in India) whereas in areas where the vector bites in the field (e.g. *P. martini* in Kenya and Uganda) this can be expected to be less effective. Because the breeding-sites of sandflies are generally unknown, control measures that act specifically against immature are not feasible. Reports of insecticide-resistance refer to only three sandfly species (*P. papatasi*, *P. argentipes* and *S. shorttii*) against DDT in one country (India), although there are reports of DDT-tolerance in several countries.

**Pathophysiology**

An important aspect of the immune system is the balance between two arms of the T-helper response. Broadly speaking, the T-helper1 (Th1) response is tailored to intracellular pathogens, such as viruses and some bacteria and parasites. Because these organisms live inside cells, they are not accessible to antibodies. The Th1 response therefore stimulates other defence mechanisms such as macrophages. The T-helper2 (Th2) system, by contrast, promotes a vigorous antibody response. The two arms are antagonistic, so a strong Th1 response means a weak Th2 response and vice versa. In leishmaniasis, where the parasites are intracellular, a strong Th1 response will kill the parasite and a strong Th2 response will lead to uncontrolled disease.

A gel produced by the *Leishmania* parasite in the gut of the sandfly prevents the insect from feeding properly. This causes more effort to feed, providing more chances for transmission of the parasite. The gel is injected into the human with the parasite and increases the severity of the infection. The crucial molecule in the gel, called filamentous proteophosphoglycan, interferes with the human immune system. The gel pushes the immune response to the non-protective T-h2 arm. The parasite thus manipulates the sandfly to make it feed more and then manipulates the host's immune system so that it can spread unchecked. Sandfly saliva is important for the establishment of infection and disease pathogenesis. The sandfly saliva contains the vasodilator maxadilan. Saliva proteins seem to influence the immune response, resulting in a shift from Th1 to Th2 response. It is possible that the age-related decrease of susceptibility to leishmaniasis is due to anti-sandfly saliva antibodies.
Life cycle, *Leishmania sp.*

The parasite’s life cycle is quite simple. When an infected sandfly bites, the parasite (as a promastigote) is injected directly into the skin. This unicellular parasite then penetrates the cells of the reticuloendothelial system (macrophages), where it multiplies in the form of amastigotes (the non-flagellate form) (“a” = without; “mastix” = whip). It is this form that can be seen in a skin biopsy or bone marrow aspirate. Multiplication results in bursting of the host cell, whereupon other cells become infected.
Leishmania amastigotes. This is the form present in human tissue. Copyright ITM

When another sandfly later bites, these infected cells can be ingested. The parasite is then still located in infected macrophages. The blood meal in the stomach is completely surrounded by a peritrophic membrane. The parasite transforms into a different form (promastigote with flagellum) in the insect and then multiplies. After 2-3 days the peritrophic membrane is digested and the parasites are released into the lumen of the stomach and intestine. They then attach to the microvilli of the intestine by means of their flagellae. They produce an enzyme, chitinase which damages the chitin coating of oesophageal-gastric junction, so that the valve between stomach and oesophagus no longer functions adequately and leaks resulting in a backflow of parasites to the mouthparts. The parasites accumulate 7 to 10 days later in the insect’s proboscis and can be injected when the insect bites its next victim. The insect is infectious 7-10 days after an infected meal and has to survive for
this time in order to be transmitted. Haemoglobin degradation products inhibit the secretion of chitinase and/or inhibit the enzyme itself making backflow of parasites to the mouthparts more difficult. Certain plant sugars do not have this effect. The insects also feed on plant juices. A balance between plant and animal feeding is required for successful transmission. A botanical description of the vector’s environment (biotope) can be important in scientific studies.

Kala azar can be transmitted in other ways, but these are exceptional, namely shared use of needles among intravenous drug users or infected blood transfusion. Very rare cases of congenital kala azar infection have been reported.

### Historical note, discovery of the parasite

The search for the origin of kala azar initially proceeded with great difficulty. Many hypotheses were investigated: for example, hookworm infection (ancylostomiasis) or malaria were thought to be responsible for the clinical condition. In 1900 an Irish soldier developed kala azar, after a stay in Dum Dum, near Calcutta, India. He died in England. The Scottish physician Dr. William Boog Leishman, later Director-General of the medical service of the British Army, carried out the autopsy. In spleen tissue he discovered small particles within the macrophages. He suspected that these were a sort of partly digested trypanosomes. A previously used name for visceral leishmaniasis was “Dum Dum fever” and refers to this historical event. The Irish physician Dr. Charles Donovan investigated splenic aspirates (needle biopsies of the spleen) from kala azar patients and confirmed Leishman’s discovery. The tiny particles were called Leishman-Donovan bodies.

### Visceral leishmaniasis - Kala Azar

#### Distribution

At present 90% of all visceral leishmaniasis occurs in India, Bangladesh, Nepal, Ethiopia, Sudan and Brazil. Visceral leishmaniasis may be responsible for 500,000 new cases and > 50,000 deaths per year.
Clinical aspects

After an initial multiplication in the skin, causing a transient small lesion the parasites can further multiply in bone marrow, liver and spleen. This causes visceral leishmaniasis. The incubation period is usually 2 to 6 months. The pathogens are usually *Leishmania donovani* and *L. infantum*. Rarely *Leishmania tropica*. *L. chagasi* is now considered identical to *L. infantum* and was possibly introduced into the New World via infected dogs or rats at the time of the Spanish and Portuguese conquests although there are doubts about this.

Visceral leishmaniasis in Southern Europe was initially considered to be a paediatric disease (hence the name *L. infantum*). However it is clear that all age groups can be infected. The disease is characterised by a persistent inflammatory state with chronic fever, enlarged liver and spleen and a low blood count (pancytopaenia = anaemia + leukopaenia + thrombocytopaenia). This must be distinguished from an aplastic bone marrow. The patient becomes very susceptible to other infections (pneumonia, tuberculosis, dysentery) which can sometimes prove fatal. Symptoms and signs of superimposed bacterial infections may confuse the clinical picture at the time of initial diagnosis. Low blood platelet counts result in a bleeding tendency (nosebleeds, bruising, etc.). Sometimes there are also other symptoms, such as swollen lymph nodes, more common in Sudan than in India. Weight loss and emaciation are frequent and substantial. The skin can turn a dark colour: kala azar (Hindi) means “black fever” and refers to this hyperpigmentation. This was mainly described from Indian cases. The reason of this hyperpigmentation is not clear. The infection can proceed atypically in HIV patients (for example without fever or splenomegaly, or with negative serology). When immunosuppression is induced by chemotherapy, latent kala azar can become clinically apparent.
Visceral leishmaniasis (kala azar) with hepatosplenomegaly. Copyright ITM

**Post- Kala azar Dermal Leishmaniasis (PKDL)**

A skin condition, called post-kala azar dermal leishmaniasis (PKDL), can occur after a patient has suffered from kala azar. PKDL rarely occurs without being preceded by kala azar. PKDL occurs on average 4-8 months after kala azar (range 0-3 years), though there are strong regional variations (in India 2-3 years after the disease, in Sudan typically within six months). This disease occurs mainly in India (up to 20% of kala azar patients) and to a much lesser extent in the Middle East. In Sudan the disease occurs regularly (56% of kala azar patients in one study). It is virtually unknown in the Mediterranean Basin or in South and Central America. It involves discoloured patches and painless nodules on the skin that usually contain few, but sometimes moderate numbers of amastigotes. Most of the lesions occur on the face (98%) and to a lesser extent on the thorax (80%), arms (70%), legs (40%), tongue (40%) and genitals (6%). This disease has a very chronic course (years) and is therefore important for transmission. Parasites do not affect internal organs in PKDL. There is sometimes a concomitant neuritis, which can further contribute to the clinical resemblance to leprosy. In East-Africa, this condition heals spontaneously in up to 80% of patients. Treatment with glucantime can be given for 2 months, or longer (4 months in India, where resistance to antimony is higher). Amphotericin B is an alternative. The therapeutic place of miltefosine for PKDL is not clear at present.

**Diagnosis**

In endemic areas, fever lasting more than 2 weeks and accompanied by splenomegaly not responding to antimalarial therapy, strongly increases the suspicion of visceral leishmaniasis, but this clinical picture is not sufficient for diagnosis.
Diagnosis of visceral leishmaniasis is not easy, as none of the tests have 100% sensitivity and 100% specificity. Clinical syndromic diagnosis lacks specificity as malaria, hyperreactive malaria splenomegaly, trypanosomiasis, typhoid fever, disseminated tuberculosis, brucellosis, haematological disorders, splenic abscess or splenomegaly due to portal hypertension all can be accompanied by enlarged spleen, fever, wasting, anaemia and/or lymphadenopathy. Because of the high cost and toxicity of current therapeutic options, empirical treatment is not advised. Therefore confirmatory diagnostic tests must be used. The leishmanin skin test is an indicator of past infection and is not used to diagnose visceral leishmaniasis.

Direct diagnosis

Direct diagnosis is made by demonstrating the presence of amastigotes (in bone marrow, spleen or lymph node aspirate). The parasite is egg-shaped and measures 2-3 x 5 µm. With Giemsa staining, there is a pale blue cytoplasm, a well-defined nucleus and a smaller kinetoplast. Microscopy requires considerable expertise and training. Usually bone marrow is obtained by sternum aspiration. The
technique of spleen aspiration is more sensitive (in some studies very nearly 100%, though in reality slightly lower) than bone marrow aspiration but can be risky (spleen rupture, haemorrhage). The platelet count should be above $40 \times 10^9$/litre. Active bleeding, severe anaemia, jaundice, moribund state, pregnancy and lack of cooperation are contra-indications. Patients must lie in bed for several hours after the procedure. Vital signs must be checked frequently to allow early recognition of haemorrhage and blood transfusion facilities must be available. To perform the procedure a 21-gauge needle and a 5 ml syringe is required. After penetration of the skin, the plunger is withdrawn, the needle is quickly inserted into the spleen while maintaining suction and withdrawn immediately (i.e. less than 1 second). Lymph node aspiration and/or liver biopsy are sometimes necessary. The parasites can rarely be detected in peripheral blood monocytes.

Serology

Serology is positive in most cases of visceral leishmaniasis. Gel diffusions immunoelectrophoresis, complement fixation test, indirect haemagglutination, Western Blot and countercurrent immunoelectrophoresis have limited diagnostic accuracy and/or feasibility in the field. Indirect fluorescence tests (IFA) are an alternative but require a fluorescent microscope. The direct agglutination test (DAT) is often used as this has a high sensitivity and specificity. Both liquid and freeze-dried antigens can be used, although liquid antigen is associated with poor reproducibility in East Africa (most likely due to decay of liquid antigen during storage and transport). Note that freeze-dried antigen does not require refrigeration. The DAT is simpler than many other tests but requires equipment, such as microplates and micropipettes, training and regular quality control. A suggested cut-off value of 1/3200 is often used but should be evaluated in each setting. An alternative is to consider titres $< 1/1600$ to be negative, borderline between 1/1600 – 1/12800, and positive $> 1/12800$. It can be defended that in a rural endemic area, a patient with more than two weeks fever and splenomegaly with strongly positive DAT values and no response to antimalarials doesn’t necessitate formal demonstration of parasites. With borderline serological values tissue aspiration with search for amastigotes will be needed. A possibility in a small regional clinic is to absorb a drop of blood from a patient suspected to have kala azar on a small filter paper and then to punch out a standard size disk from the blood spot. In this way one obtains a well-defined, accurate aliquot of absorbed blood. This can be transported and used for DAT in a well equipped laboratory. Serology remains positive after cure. The fast agglutination screening test (FAST) is a simplified (single serum dilution) and more rapid version of the DAT (2-3 hours versus 18h). Because DAT is not practical in many field conditions alternatives are being studied. ELISA is highly sensitive, but specificity depends upon the antigen used (amastigotes or promastigotes). Recombinant K39 antigen-based dipsticks using immunochromatography (ICT) have been an important step forward and have replaced DAT as first line test. K39 is a 39-amino acid repeat that is part of a kinesin-related protein of L. chagasi. This
repeat is conserved within the *L. donovani* complex. The ICT tests are easy to perform, rapid and cheap. Twenty µl of serum are added on the dipstick, which is then placed vertically in a test tube. Two drops of chase buffer solution provided with the dipstick are then added. The results are read after 5 to 10 minutes. Even a weak band in the test region is considered positive. A control line has to be visible. It is the most promising tool for the diagnosis of visceral leishmaniasis in peripheral centres. The specific format (brand) of dipstick may play an important role (e.g. Opti-Leish™, DiaMed IT Leish™, DiaMed DUAL IT L/M™ versus Kalazar Detect™).

Formol-gel test.

In kala azar there is a very high production of non-specific immunoglobulins (and a decrease in albumin), especially in advanced disease (i.e. more than 3 months). This can be demonstrated by serum protein electrophoresis, but this impractical in field conditions. The proteins can be precipitated as a gel by formalin. Twenty µl of 40% formaldehyde are added to 200 µl of serum in a glass tube. After twenty minutes, the gelification reaction is visually assessed as positive or negative. The test is simple and cheap. The test can also be positive in patients with hyperreactive malaria splenomegaly.

Katex

A urinary antigen detection test using latex agglutination (KAtex) has been developed to circumvent the limitations of serological tests. It detects a heat-stable low molecular weight carbohydrate antigen. This will become negative upon successful treatment. It can therefore distinguish an active from a past infection. A very high specificity and moderate to high sensitivity were reported. The test requires the boiling of 1 ml of urine for 5 minutes. About 50 µl of the treated urine sample is added onto a reaction zone on a glass slide and a drop of latex is added. The liquids are stirred to a completely homogenous mixture. Any agglutination reaction discerned when compared with a negative control is considered positive. The sensitivity varies with the parasite load.

Culture

Culture can be done from peripheral blood, buffy coat or tissue aspirates. The microculture method improves sensitivity and decreases incubation periods. Cultures are expensive, time-consuming and require expertise. A *Leishmania* parasite can survive for 3 days at a temperature of 4° C, but for only 1 day at room temperature, in Locke transport medium (a buffered glucose-salt solution with antibiotics).

Genome assays
Lack of standardisation and quality control is a major concern of PCR and related assays. A multitude of gene targets, protocols and applications have been described. A PCR assay was developed in order to amplify the kinetoplast minicircle of *Leishmania* species (it can be also be used in vector studies). The kinetoplast minicircle is an ideal target because it is present in 10,000 copies per cell and its sequence is known for most *Leishmania* species. The very high sensitivity of PCR-based assays may actually be a disadvantage by being a marker of infection (transient or permanent) instead of being a marker of disease, as it will pick up also asymptomatic carriers. Detailed genomic analysis of *L. donovani* showed that parasites can have two, three, four or even five sets of chromosomes in one organism. Further study of this ploidy-variation will investigate the possible clinical implications of this unexpected finding.

**Montenegro test**

Leishmanin is a compound obtained via in vitro culture of promastigotes. A skin test with leishmanin (Montenegro test) is negative during active kala azar, but later becomes positive (after 6 to 12 months). The Montenegro test reflects the suppressed cellular immunity during infection. There is a specific anergy for *Leishmania* parasites during active disease. This test is mainly of epidemiological value. To perform the test 0.1 ml is injected intradermally and the local reaction read after 48 hours (>5 mm induration = positive). A positive test eliminates the existence of active kala azar. Cutaneous leishmaniasis produces a positive Montenegro test.

**Treatment of VL**

Pentavalent antimonial compounds.

One of the treatment options for visceral leishmaniasis are pentavalent antimony derivatives (antimony, chemical symbol Sb = Stibium). The derivative most frequently used is Glucantime® (meclumine antimonate, 85 mg Sb/ml) and rarely Pentostam® (sodium stibogluconate, 100 mg Sb/ml). The drugs can be administered IM (intramuscularly, painful) or by slow IV (intravenous) injection or infusion (diluted with 5% glucose solution, otherwise local thrombophlebitis occurs). The dose is always expressed as mg Sb: 2 x 10 mg/kg IM or slow IV infusion per day for at least 30 days. On an ampoule might be written 1500 mg/5 ml, which is 1500 mg calculated as the salt, not as stibium itself. This can lead to underdosing if one is not aware of this detail. As a dose is practically totally excreted and eliminated via the urine within 6 hours after administration, a twice daily administration would pharmacokinetically be more logical than an injection once daily. However, a single administration per day appears to suffice in practice. The dose should be reduced in patients with kidney failure. A maximum of 850 mg/day [10 ml Glucantime®] has been previously set due to
the risk of cardiotoxicity with higher doses. This limit has been contested and has been abandoned in the latest WHO guidelines. T-wave inversion and prolongation of the QT-time are indicative of threatening arrhythmia. The fever usually disappears after 1 week. The spleen begins to get smaller after 2 weeks but frequently requires 6 to 12 months to return to normal.

**Antimony**

Antimony is just below arsenic in the periodic table. It mimics the toxic effects of arsenic, which result from binding to adjacent thiol groups on enzymes, thereby impairing their function. Antimony is found in trivalent and pentavalent forms. Inhalation of stibine gas (SbH₃) causes massive haemolysis. Pentavalent antimonials (e.g. meglumine antimoniate, sodium stibogluconate) are used for treatment of leishmaniasis. One of their actions is to inhibit phosphofructokinase, the rate-limiting step in the parasites’ glycolytic pathway.

Follow-up and response in the event of recurrence

Follow-up is necessary as a number of patients will relapse. This usually happens in the first 6 months after treatment. Upon recurrence of visceral leishmaniasis (relapse), higher doses of Glucantime® can be used for a longer time (2-3 months). Alternatively and preferably another drug or combination therapy can be used to treat relapses.

Cases of complete treatment unresponsiveness can occur. Splenectomy sometimes has to be carried out in cases of life-threatening anaemia or thrombocytopenia. If possible pneumococcal vaccination should be given before the operation, and lifelong antimalarial prophylaxis is indicated thereafter if the patient stays in an endemic area.

Alternative treatments:

While antimonials have been the mainstay for treatment of visceral leishmaniasis for many decades, alternative options have been explored, mainly driven by the emergence of antimonial resistance in India, but also by their toxicity. Currently first line drugs entail antimonials, conventional amphotericin B and the lipid-containing formulations, paromomycin and miltefosine.

**Amphotericin B** is a polyene and has a fairly complex structure with a hydrophilic and a lipophilic component. The recommended dose of amphotericin B [Fungizone®] is 0.5-1 mg/kg/day IV, to be given over 6 hours; total dose max. 1-3 g. This drug is mainly used for the treatment of deep
mycoses, though it is also active against *Leishmania*. It is a rather toxic medication. Shivering, fever, nausea, vomiting, headache, anaemia, phlebitis at the site of the infusion, cardiotoxicity, kidney failure, hypokalaemia and hypomagnesaemia are frequent side effects. Side effects occurring shortly after administration can be reduced by cortisone IV or meperidine (pethidine), a morphine analogue. Administration of 500-1,000 ml physiological isotonic saline solution before starting the IV-drip reduces the risk of nephrotoxicity. The toxicity of the drug is reduced by pharmacological complexing with lipids prior to the administration. The drugs are then concentrated in the reticuloendothelial system and not in the kidneys so that a higher daily dose per kg of bodyweight can be administered and treatment time shortened (e.g. to 5 days). There are good indications that single-dose treatment (high dose; 10 mg/kg of the liposomal formulation) is useful, at least in the Indian subcontinent (India, Nepal, Bangladesh). In 1990 AmBisome® was developed as a first-choice drug. Several lipid formulations of amphotericin B are now available. They differ from each other in the type of phospholipid and the ratio of lipid to amphotericin B. Good results have been obtained with these lipid formulations. The price of these medications (AmBisome®, Amphotec®, Abelcet®) has come down, but is still high for the average rural farmer in a developing country.

**Formulations of Amphotericin B**

1. **Fungizone®:** Amphotericin B deoxycholate. Contains no lipids.
2. Emulsification of Fungizone® in Intralipid 20%; little reduction of toxicity
3. **AmBisome®:** L-AmB: incorporation in liposomes (vesicles).
4. **Abelcet®:** ABLC or Amphotericin B Lipid Complex. Microscopically small ribbon-like membranes formed by complexing with phospholipids.
5. **Amphotec®:** ABCD (= Amphocil®) Amphotericin B Colloidal Dispersion: AmB-cholesteryl sulphate forms disc-shaped structures.

**Injectable aminosidine** (paromomycin) is now also a first line drug. It is an aminoglycoside antibiotic. In 2007 the results of an Indian study showed that paromomycin IM, at a dose of 11 mg/kg/day x 21 days was noninferior to amphotericin B at a dose of 1 mg/kg IV every other day x 30 days. The combination with antimonials for 17 days was also found effective in East-Africa. Pain at the injection site, liver toxicity and ototoxicity were reported as side effects. Paromomycin for IM administration is licensed in India, and since 2012 also in Nepal. Combined with antimonials it is the first line regimen in East-Africa.

**Miltefosine** (Miltex®) was approved for use in India in 1992. It became more widely became available in subsequent years. Miltefosine or hexadecylphosphocholine is a lecithin analogue (=phosphatidyl-choline analogue). In the molecule phosphatidylcholine is bound to a carbohydrate
component via an ether bridge instead of an ester. Miltefosine interferes with certain cellular signal cascades and with membrane synthesis, though its precise mode of action is still unknown. It was initially developed as an antineoplastic agent. In the 1990s it was also discovered that in vitro and in animal models it was active against *Leishmania* parasites. These organisms contain many ether lipids in the cell membrane. The main advantage of the compound is that it can be given orally, in contrast to the injectable antimony derivatives and amphotericin B. It cannot be given IV as this would lead to haemolysis. The molecule is fairly easy to produce and this should eventually bring down the price, which is quite high in the West. The daily dose for adults is 100-150 mg, and for children 2.5 mg/kg/day. It should be given for 4 weeks. The half-life is several weeks. The cure rate was high in studies in India, although lower efficacy was found in East-Africa. Dose-dependent gastrointestinal discomfort often occurs and reversible hepato- and nephrotoxicity sometimes occurs. It is teratogenic and so cannot be given to pregnant women or women who want to conceive in 6 months after treatment. How quickly resistance to miltefosine will develop when used as monotherapy in the field is not yet clear. It is relatively easy to induce resistance in vitro. In this regard, it is of concern that success rates have been declining over the last years in the Indian subcontinent, although it is not yet well defined whether this relates to true parasite resistance, underdosing or evolving parasite fitness are also considered as alternative explanations. This has led to the use of liposomal amphotericin B (AmBisome) as first line treatment in the Indian subcontinent.

**Combination therapy** This is the suggested way forward to increase treatment efficacy, prevent the development of drug resistance, reduce treatment duration and possibly decrease cost. Pentavalent antimonials combined with paromomycin is now first line treatment in East-Africa. Other combinations including liposomal amphotericin B, paromomycin and miltefosine were found effective in India in phase III trials. Phase IV studies are ongoing.

**Table: The main drugs currently used for treatment of visceral leishmaniasis.**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Regimen</th>
<th>Marketing</th>
<th>Clinical efficacy</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentavalent antimonials</td>
<td>20 mg/kg iv or im daily for 28-30 days</td>
<td>Albert David (SSG); GSK (Pentostam®); Sanofi Aventis (Glucantime®)</td>
<td>35-95% (depending on geographic area)</td>
<td>As high as 60% (Bihar, India)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.75-1 mg/kg iv for 15-20 doses (daily or alternate days)</td>
<td>Bristol Meyers Squibb (Fungizone®); Generic companies</td>
<td>&gt; 97% all regions</td>
<td>Not documented</td>
</tr>
</tbody>
</table>
### Liposomal Amphotericin B
- **Dosage:** 10-30 mg/kg total dose iv; usually 3-5 mg/kg/dose single dose (10 mg/kg) in India
- **Manufacturer:** Gilead (AmBisome®)
- **Region:** Europe and Asia: > 95%; Africa: not fully established (higher dose required?)
- **Issues:** Not documented

### Miltefosine
- **Dosage:** 2-2.5 mg/kg/d orally daily over 28 days (India only)
- **Manufacturer:** Paladin (Impavido®)
- **Region:** Asia: 94% (India) Africa: single field study (93% in HIV(-))
- **Issues:** Readily obtained in lab isolates

### Paromomycin sulphate
- **Dosage:** 15 mg/kg im daily for 21 days (India only)
- **Manufacturer:** IOWH/Gland Pharma
- **Region:** Asia: 95% (India) Africa: 15 mg/kg: 64% (Sudan <50%) 20 mg/kg: 80% (Sudan)
- **Issues:** Readily obtained in lab isolates

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*a marketing authorization holder iv: intravenous; im: intramuscular; SSG: sodium stibogluconate

**Table: The main drugs currently used for treatment of visceral leishmaniasis (continued).**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Toxicity</th>
<th>Cost/course</th>
<th>Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentavalent antimonials</td>
<td>Frequent, potentially severe; Cardiac toxicity, Pancreatitis, Nephro + hepatotoxicity</td>
<td>Generic ~ $53</td>
<td>Quality control; Length of treatment; Painful injection; Toxicity; Resistance in India</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Branded ~ $70</td>
<td></td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>Frequent Infusion-related reactions, Nephrotoxicity (in-patient care needed)</td>
<td>Generic price: ~ $21</td>
<td>Need for slow iv infusion; Dose-limiting; Nephrotoxicity; Heat stability</td>
</tr>
<tr>
<td>Pharmacological Agent</td>
<td>Adverse Effects</td>
<td>Price Notes</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td>------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Liposomal Amphotericin B</td>
<td>Uncommon and mild; Nephrotoxicity (limited)</td>
<td>Preferential price: $280 (20mg/kg total dose) Commercial price: ~ 10x Price; Need for slow iv infusion; Heat stability (stored &lt;25° C)</td>
<td></td>
</tr>
<tr>
<td>Miltefosine</td>
<td>Common, usually mild and transient; gastrointestinal (20-55%), Nephro + hepatotoxicity Possibly teratogenic</td>
<td>Preferential price: ~ $74 Commercial price: ~ $150 Price; Possibly teratogenic; Potential for resistance (half-life); Patient compliance</td>
<td></td>
</tr>
<tr>
<td>Paromomycin sulphate</td>
<td>Uncommon, Nephrotoxicity Ototoxicity Hepatotoxicity</td>
<td>~ $15 Efficacy variable between and within regions; Potential for resistance (?)</td>
<td></td>
</tr>
</tbody>
</table>

Table. Treatment recommendations for visceral leishmaniasis per geographical region, as recommended by the WHO (in order of preference)

### L Donovani - Indian subcontinent

1. Liposomal amphotericin B: 3-5 mg/kg/d iv over 3-5 days for total dose of 15 mg/kg or 10 mg/kg iv sd

2. Combination regimens (sequential co-administration)

   Liposomal amphotericin B (5 mg/kg iv sd) + miltefosine (dosage as below) for 7 days

   Liposomal amphotericin B (5 mg/kg iv sd) + paromomycin (dosage as below) for 10 days

   Paromomycin + miltefosine (dosages as below) for 10 days

3. Amphotericin B deoxycholate 0.75-1 mg/kg/d iv, daily or on alternate days, for 15-20 doses

4. Miltefosine: children 2-11 years: 2.5 mg/kg/d; ≥12 years and < 25 kg body weight: 50 mg/day; 25-50 kg: 100 mg/day; > 50 kg: 150 mg/day; orally for 28 days

5. Paromomycin 15 mg (11 mg base)/kg/d im for 21 days
6. Pentavalent antimonials: 20 mg Sb\(^{5+}\)/kg/d im or iv for 30 days in areas where they remain effective (including Nepal, Bangladesh and certain areas in India)

7. Rescue treatment in case of non-response: conventional amphotericin B deoxycholate or liposomal amphotericin B at higher doses

**L Donovani - East-Africa**

1. Combination therapy: pentavalent antimonials + paromomycin for 17 days (dosages as above)

2. Pentavalent antimonials monotherapy as above

3. Liposomal amphotericin B 3-5 mg/kg/d iv over 6-10 days for total dose of 30 mg/kg

4. Amphotericin B deoxycholate as above

5. Miltefosine as above

**L infantum**

1. Liposomal amphotericin B 3-5 mg/kg/d iv in 3-6 doses for a total dose of 18-21 mg/kg

2. Pentavalent antimonials 20 mg/kg Sb\(^{5+}\)/kg/d im or iv for 28 days

3. Amphotericin B deoxycholate 0.75-1 mg/kg/d iv, daily or on alternate days for 20-30 doses, total dose of 2-3 g

iv: intravenous; im: intramuscular; sd: single dose

The nitroimidazole: **fexinidazole** has potential as a safe and effective oral drug therapy for treatment of visceral leishmaniasis (see also treatment of Human African Trypanosomiasis). Both metabolites of fexinidazole (sulfone and sulfoxine) were active against Leishmania donovani amastigotes. Reliance on a single enzyme for prodrug activation may leave fexinidazole vulnerable to the emergence of drug resistance. Clinical studies are ongoing. One option under exploration in East-Africa is the combination of miltefosine and fexinidazole (both given orally).

Several other drugs mentioned below have also been explored historically but have not made it to first line treatment.
**Pentamidine isethionate** (4 mg/kg every 48 hours IM for 4 months).

**Combination therapy with gamma-interferon** was explored, based on the importance of Th1 immunity in achieving control of visceral leishmaniasis, but efficacy was only modest.

**High-dose allopurinol** (Zyloric®), e.g. 3 x 7 mg/kg/day (that is, 20 mg/kg/day), for 4-12 weeks was also effective in clinical studies.

**Terbinafine** (Lamisil®) is an antimycotic drug with some clinical activity.

**Sitamaquine.** Due to relatively low efficacy rates and safety issues, this has largely been abandoned.

**Pamidronate** a bisphosphonate drug typically used in the treatment of osteoporosis is effective against experimental cutaneous leishmaniasis. Several bisphosphonates have significant activity against *Leishmania donovani* in vitro, and several are potent inhibitors of bone resorption and in clinical use for the treatment of osteoporosis and Paget’s disease. It is possible that currently approved clinical regimens of the drug are not high enough to cure human cutaneous leishmaniasis. Pamidronate could be a useful lead compound in the synthesis of new drugs against this disease.

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**Cutaneous leishmaniasis**

**Distribution**

Approximately 90% of all cases of cutaneous leishmaniasis now occur in Iran, Syria, Saudi Arabia, Afghanistan, Algeria, Peru and Brazil.

**Clinical aspects**

Various forms are clinically distinguished, the most important of which are:

1. Localised cutaneous leishmaniasis: skin ulcers that heal very slowly or nodular lesions, limited in extent and number. These chronic sores have regional names: clou de Biskra in Algeria and Aleppo boil in Syria.
2. Diffuse cutaneous leishmaniasis: cutaneous nodules and plaques that do not ulcerate but sometimes spread over the entire body.

3. Recurrent cutaneous leishmaniasis

“... After it is cicatrised, it leaves an ugly scar, which remains through life, and for many months has a livid colour. When they are not irritated, they seldom give much pain... It affects the natives when they are children and generally appears in the face, though they also have some on their extremities... In strangers, it commonly appears some months after their arrival. Very few escape having them, but they seldom affect the same person above more than once.”

Skin ulcer due to cutaneous leishmaniasis.
Localised cutaneous leishmaniasis

After a bite by a sandfly infected with *L. tropica* (mainly urban infection), there is an incubation period of a few weeks or months, occasionally years. There is initially a small papule and usually only a single lesion, though sometimes there are several. This slowly spreads and can remain completely dry, become warty or nodular or develop into a painless, sharply delineated ulcer surrounded by a purplish raised border. Satellite lesions can occur. Spontaneous healing often occurs after 6 to 12 months, resulting in a depressed scar. Recurring cutaneous lesions – possibly with severe disfigurements – occasionally occur. There is usually immunity to any subsequent infection with the same organism. In infection with *L. major* (mainly rural infections, particularly from a rodent reservoir) the lesions are usually larger and develop more quickly, hence the name. There is a greater tendency to local spreading via the lymphatics and this has to be distinguished from sporotrichosis. The lesions will eventually spontaneously heal with scar formation. Clinical cure starts when macrophages become activated and start killing amastigotes. This is mediated via a T-helper cell type 1 (Th1) response. This immune reaction also prevents recrudescence of latent chronic infection. The Th1 response is accompanied by secretion of pro-inflammatory cytokines, such as interferon gamma and interleukin 12. If the immune response would be towards production of down-regulating cytokines (interleukin 4, 10, 13, TGF beta), macrophages will not be capable of eliminating the parasites, but tissue destruction will be limited.

In South America the lesions often have their own local names and clinical expressions. Hence in Peru they are called “uta” (a solitary ulcer or a few restricted lesions brought about by *L. peruviana*, frequently on the face). In Guyana they are known as “bush yaws” or (French) “pian bois” (*L. guyanensis*) with raspberry-like lesions that resemble yaws. In Yucatan, Mexico an ulcer on the ear (usually caused by *L. mexicana*) is known as “chiclero” ulcer.

A “chiclero” is a man who collects chicle-latex in the forest. During their activity in the plantations the workers can get bitten by *Lutzomyia olmeca* and as such are exposed to a high risk of contracting leishmaniasis, hence the term “chiclero ulcer”.

Diffuse cutaneous leishmaniasis. Infection with *Leishmania aethiopica*. Copyright ITM
Diffuse cutaneous leishmaniasis

Diffuse cutaneous leishmaniasis is a diffuse affliction of the skin with extensive non-ulcerative nodules and is a very chronic disease. It is sometimes followed by chronic lymphoedema of an affected part of the body. This disease is poorly understood but is probably caused by a diminished resistance to the parasite. This immunosuppression is possibly brought about by the parasite itself. One of the supposed mechanisms of escape of Leishmania parasites is downregulation of the expression of major histocompatibility complex (MHC) class II molecules on the macrophages they colonise. In East Africa diffuse cutaneous leishmaniasis is often caused by L. aethiopica and in the New World frequently by L. mexicana.

If there are generalised cutaneous lesions the condition must be differentiated from lepromatous leprosy, keloids, neurofibromatosis and post kala azar dermal leishmaniasis (PKDL). Due to the low
resistance of the patient very numerous amastigotes are present and most skin smears are positive. Treatment is difficult as the patient’s immune system itself is functioning poorly. DCL patients are anergic to leishmanial antigen. Patients with DCL have a predominantly Th2-type cytokine response. They have low concentrations of interferon gamma and interleukin 12. There is no tendency to self-cure. Differentiation from PKDL is important, as the latter can still be treated reasonably well. In Sudan 1 case of diffuse cutaneous leishmaniasis is found for every 100 cases of localised cutaneous leishmaniasis. The incidence varies greatly from district to district. It occurs frequently in South America, but in contrast to this it does not occur in India (or very exceptionally –eg in HIV patients).

Recurring cutaneous leishmaniasis

Recurring cutaneous leishmaniasis seldom occurs (Iraq, Iran). This disease, also known as leishmaniasis recidivans leads to significant tissue damage. Parasites are very difficult to detect in these very chronic lesions. Differentiation from cutaneous tuberculosis is important.

**Diagnosis of cutaneous leishmaniasis**

Attempts should be made to detect the parasite microscopically in a biopsy or smear from the edge of the wound. The biopsy should if possible, be divided up for pathology (seldom available, not very sensitive and is principally used more for exclusion of another cause) and cultures (bacteria, mycobacteria, fungi, *Leishmania*) and an impression preparation should also be made. Lesions on the face can be injected with 0.1 ml physiological saline and aspirated again while moving a small, thin needle back and forth in the skin. Serology is usually negative. Differential diagnosis includes ulcers due to mycobacteria, cutaneous diphtheria, tertiary syphilis, yaws, cutaneous carcinoma and deep or subcutaneous mycosis. Field sore (cutaneous diphtheria) and tropical ulcers are painful, particularly in the early phase.

Differential diagnosis of disseminated nodular and ulcerated lesion includes leishmaniasis, sporotrichosis, atypical mycobacteria and nocardiosis.

**Treatment**

The response to treatment varies according to the species. Drugs for systemic and topical treatment can be used. There is an urgent need for better and cheaper drugs.

**Indications for local treatment**
1. lack of risk of developing mucosal lesions
2. Old World cutaneous leishmaniasis
3. small, single lesion
4. absence of spread to lymph nodes

**Indications for systemic treatment**

1. presence of mucosal lesion or spread to lymph nodes
2. New World cutaneous leishmaniasis, except localised Leishmania mexicana infection
3. lesions unresponsive to local treatment

**Overview topical treatment of cutaneous leishmaniasis**

1. physical methods: cryotherapy (liquid nitrogen) for 15-20", repeated 2-3 times with an interval of e.g. 3 weeks. Blistering will occur.
2. application of local heat via a CO₂ laser or an infrared lamp (40°C to 42°C for 12 hours) has been used, but heat-induced skin bullae are common.
3. ointment with 15% paromomycin and 12% methylbenzethonium chloride in soft white paraffin (e.g. Leishcutan® ointment). Urea can be added as a keratolytic. Twice daily application is advised for a duration of 20-30 days.
4. skin infiltration with pentavalent antimony with a fine gauge needle. Blanching of the lesions should be obtained. Treatment is repeated every 5-7 days, in general 2-5 times, sometimes more.
5. imiquimod crème (Aldara®). This immunomodulator activates macrophage killing of Leishmania amastigotes, but is best used in combination with systemic meglumine antimonate. Experience with this drug is limited.
6. treatment with antimonium plus topical recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) has been described. GM-CSF (molgramostim = Leucomax®) was diluted for topical use to a concentration of 10 µg/ml. It was applied 3 times weekly for 3 weeks (1-2 µg/cm²/lesion). In vitro, GM-CSF has been shown to activate macrophages that kill Leishmania pathogens. Intralesional injection of GM-CSF (400 µg) has also been shown to reduce the healing time of leishmania ulcers.
7. Application of topical 5-aminolaevulinic acid (a porphyrin-precursor), followed by two laser irradiations, which photoactivates the compound. It is expected that very little scar tissue would form, so for aesthetically important places, this might become first choice treatment, if the clinical studies confirm this expectation.
Overview systemic treatment of cutaneous leishmaniasis

1. Pentavalent antimonials (mebumine antimoniate [85 mg Sb/ml, IM] or sodium stibogluconate [100 mg/ml, IM or filtered IV] can be given parenterally for extensive skin lesions. For unknown reasons, the incidence of herpes zoster is increased about 10 times during IV treatment with IV Pentostam relative to the incidence in the normal population. Cases of cutaneous leishmaniasis not treated with antimony do not have an increased incidence of herpes zoster.

2. Pentamidine. First line against *L. guyanensis* (French Guyana). Several treatment schemes exist and the cure rate is dose-dependent. Some short-courses use 1200 mg as a total dose. In Guyana 3 mg/kg/day every other day is often used (4 injections).

3. Imidazoles, triazoles. Infections caused by *L. major* can be successfully treated with oral fluconazole 200 mg/day for 6 weeks (cure rate of 80%). Ketoconazole 600 mg per day x 28 days is moderately effective for *L. mexicana*, but much lower against *L. braziliensis*. Treatment with ketoconazole is sometimes complicated by hepatotoxicity, abdominal pain and nausea. Itraconazole (Sporanox®) gave good results in initial studies but this was not seen in the field.

4. Miltefosine. Not yet widely available, but allows oral therapy.

5. Amphotericin B and its liposomal formulations (IV).

6. Allopurinol. Not as monotherapy, but in combination with e.g. pentavalent antimony for *L. panamensis*.

Treatment of diffuse cutaneous leishmaniasis (*L. aethiopica*)

The treatment of diffuse cutaneous leishmaniasis caused by *L. aethiopica* is problematical, as this parasite is less sensitive to Glucantime®. Pentamidine can be used against *L. aethiopica*. A dose of 4 mg/kg/week which has to be continued for at least 4 months after disappearance of the parasites from the skin is an acceptable guideline here. Parenteral aminosidine sulphate is another therapeutic possibility. This is an antibiotic that is obtained from *Streptomycyes chrestomyceticus*. It is an aminoglycoside and is thus potentially nephro- and ototoxic. It is chemically identical to paromomycin, which is obtained from a related *Streptomycyes* strain. The compound is not resorbed from the intestine. Recurrences are frequently seen with aminosidine given as monotherapy. Aminosidine is however synergistic with stibogluconate and a permanent remission can be obtained with the combination of aminosidine with Glucantime® or Pentostam®. The dose is 14 mg/kg/day IM to be continued for up to 60 days after all parasites have been eliminated. The total treatment period takes 6 months or more. Good results were obtained with amphotericin B.
Mucocutaneous leishmaniasis

Distribution

At present 90% of all mucocutaneous leishmaniasis occurs in Bolivia, Peru and Brazil. Illustrations of skin lesions and disfigurements suggestive of leishmaniasis are encountered on pre-Inca earthenware. These indicate that the disease was already in existence in Peru and Ecuador in the 1st century AD. Texts dating from the 15-16th century Inca period and the Spanish conquest mention the risk of cutaneous ulcers in seasonal farmers. Espundia was also described as “white leprosy”.

Clinical aspects

When skin and mucosae are affected the disease is known as mucocutaneous leishmaniasis. This is very rare in East Africa but frequent in South America, where it is known as “espundia”. After an initial skin lesion, that slowly but spontaneously heals, chronic ulcers appear after months or years on the skin, mouth and nose, with destruction of underlying tissue (nasal cartilage, for example). Tissue destruction with disfigurement can be very severe. Parasites are usually rare in the lesions. A substantial part of the disfigurement is possibly due to immunological mechanisms. One hypothesis is a relationship between the occurrence of mucocutaneous lesions and the presence of certain alleles of polymorphic tumour necrosis factor a and b genes.
Espundia or mucocutaneous leishmaniasis often results from infection with Leishmania brasiliensis.
Photo Cochabamba, Bolivia
Espundia or mucocutaneous leishmaniasis often results from infection with Leishmania braziliensis. Photo Cochabamba, Bolivia

**Diagnosis**

The lesions often contain few parasites. Diagnosis is sometimes made solely on a clinical basis. Culture of the parasites is possible, but not really feasible in primitive rural conditions. Serology in espundia can be positive or negative (the quality of the antigen is of crucial importance). A practical problem in South America is whether a certain skin lesion with *Leishmania* amastigotes is caused by *L. braziliensis* or not. The geographical origin of the lesion or PCR and/or zymodeme analyses may give an answer here, though these laboratory techniques are not available in rural areas.

**Mucocutaneous leishmaniasis, differential diagnosis:**
Differential diagnosis includes skin cancer, tertiary syphilis and yaws, leprosy, rhinoscleroma (a very chronic granulomatous infection with *Klebsiella rhinoscleromatis*), rhinosporidiosis, midline granuloma (a form of T-cell lymphoma), Wegener’s granulomatosis, sarcoidosis, skin tuberculosis, infection with the free-living amoeba *Balamuthia mandrillaris*, chronic nasal cocaine abuse, noma, and fungal infections such as cryptococcosis, histoplasmosis and South American blastomycosis (paracoccidioidomycosis). With this last disease, which is a very chronic infection, the lungs are frequently affected in a manner that can mimic tuberculosis. The yeast has typical oval cells with ectospores and can be detected in sputum.

**Overview: Differential diagnosis of nasal ulcers:**

1. Mucocutaneous leishmaniasis (espundia)
2. Fungal infections, such as paracoccidioidomycosis (syn. South American blastomycosis), histoplasmosis, cryptococcosis, coccidioidomycosis
3. Actinomycosis
4. Treponematoses (syphilis, yaws, bejel)
5. Leprosy
6. Tuberculosis
7. Rhinosporidiosis
8. Rhinoscleroma (chronic infection with *Klebsiella rhinoscleromatis*)
9. Balamuthiasis (infection with free-living amoeba)

**Non-infectious**

1. Granulomatosis with Polyangitis (formerly Wegener granulomatosis)
2. Midline granuloma (a form of T-cell lymphoma)
3. Other non-Hodgkin lymphoma
4. Squamous cell carcinoma
5. Sarcoidosis
6. Relapsing polychondritis
7. Cocaine abuse

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Trypanosomiasis

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Human African trypanosomiasis

Summary

- Difference between Gambian (western) and Rhodesian (eastern) trypanosomiasis
- Restricted to well defined regions in Africa, determined by tsetse fly vectors
- Early/first stage: transient sore, fever, oedema, lymphadenopathy, splenomegaly
- Late/second stage: central nervous system symptoms with abnormal CSF (elevated cells and protein, Mott cells, trypanosomes)
- Diagnosis: always try to detect the parasite
- Repeated thick smears, Buffy coat, Woo technique, mAECT, lymph node aspiration
- When parasite found in blood or lymph node, always lumbar puncture to determine the stage
- Indirect: serology (CATT for West African form), clinical evidence
- Difficult treatment depending on species and stage: pentamidine, suramin, melarsoprol, eflornitine and nifurtimox
- Currently nifurtimox-eflornithine combination therapy (NECT) in first-line against second-stage T. b. gambiense trypanosomiasis. Fexinidazole oral short course.
- Importance of early diagnosis and follow-up as well as integration of control in primary care.

General

Human African trypanosomiasis (HAT) is caused by infection with a unicellular parasite. There are two subspecies of these parasites: the West African or Trypanosoma brucei gambiense and the East African or T. brucei rhodesiense. They cannot be differentiated from each other on morphological grounds. T. brucei gambiense has two subtypes, T. brucei gambiense type 1 and 2. The main difference resides in their ability to avoid the uptake or (T. b gambiense type 1) or to neutralize/compensate (T. b gambiense type 2) the trypanosome lytic factor, a human serum component. T. b. gambiense type 2 resembles T. b. brucei, an animal infecting trypanosome and causes a more acute disease than type 1 T. b. gambiense.
Transmission takes place through the bite of an infected tsetse fly (Diptera, genus *Glossina*). Since the parasites are transmitted via tsetse saliva, they are also known as “salivaria”, as opposed to *Trypanosoma cruzi*, which belongs to the “stercoraria” because of its transmission via the feces of the kissing bug. In exceptional cases, mechanical transmission takes place via other biting flies (tabanids). Congenital infections are rare. Sexual transmission seems to be extremely rare.

**Epidemiology**

African trypanosomiasis occurs exclusively in sub-Saharan Africa, with its distribution being defined by the tsetse fly occurrence. Because of its clinical presentation, the West African form is also called sleeping sickness. The area of distribution lies between 14° north of the Equator and 29° south of the Equator. The areas of distribution of West African and East African trypanosomiasis show little overlap. Most of the endemic countries have only one form of the disease: the Western form, or the Eastern form. This facilitates national therapeutic guidelines. However, both West and East African trypanosomiasis exist in Uganda. Both forms have their own foci, but these are now converging in Uganda. They did not overlap in 2015, but are separated now by only a narrow corridor of about 100 km. If the transmission areas meet (as feared), it would considerably complicate diagnosis and guidelines for management of clinical cases in Uganda.

There have been several large epidemics in Africa in the last 120 years. One from 1896 till 1906 mostly in Uganda and the Congo Basin. Numbers were skyrocketing in many African countries in 1920 but by the mid-1960s, the disease was under control with less than 5000 cases reported in the whole continent, thanks to mobile teams which carried out the screening of millions of people at risk. After this success, surveillance was relaxed, and the disease reappeared, reaching epidemic proportions in several regions by 1970. In 1998, almost 40,000 cases were reported, but estimates were that 300,000 cases were undiagnosed and therefore untreated. In the last decades of the 20th century, prevalence reached 50% in several villages in Angola, the Democratic Republic of the Congo, and South Sudan. Sleeping sickness was the first or second greatest cause of mortality in those communities, even ahead of HIV/AIDS.

The efforts of WHO, national control programmes, bilateral cooperation and nongovernmental organizations (NGOs) during the 1990s and early 21st century reversed the curve. In 2009, after continued control efforts, the number of cases reported dropped below 10,000 (9,878) for the first time in 50 years. This decline in number of cases has continued with 997 new cases reported in 2018, the lowest level since the start of systematic global data-collection 80 years ago. The estimated population at risk today is 65 million people. The area reporting ≥ 1 case/10,000 inhabitants/year in the five-year period (2012–2016) has shrunk by 61% from the baseline period (2000–2004). Since the
number of new human African trypanosomiasis (HAT) cases reported between 2000 and 2018 dropped by 95%, the WHO neglected tropical diseases road map targeted its elimination as a public health problem (< 1 case/10,000 inhabitants/year) by 2020 and interruption of transmission (zero cases) for 2030.

To achieve complete elimination of HAT, the main challenge is to set up a cost-effective, adapted and sustained HAT control and surveillance strategies. Integration of the vertical HAT control activities in the general health system will be needed, which is often particularly difficult in those peripheral rural areas where the disease is more entrenched and the health system is weak. Sustained commitment of donors will be crucial. The role human asymptomatic carriers, of parasites in the skin, and by the possible animal reservoirs in gambiense HAT epidemiology, will be essential.

**Countries reporting cases anno 2019:**

*T. b. gambiense*: Guinea, Equatorial Guinea, Nigeria, Cameroon, Gabon, Chad, Central African Republic, Congo, DR Congo, Angola, South Sudan, Uganda. Countries with historical *T. b. gambiense* HAT with surveillance activities not reporting any cases are Benin, Ivory Coast, Mali, Niger, Senegal, Sierra Leone and Togo. This anthroponotic subspecies affects mainly humans but is sometimes isolated in pigs, dogs, ... The role of animals in transmission is unknown but probably very limited.

Between 1999 and 2019, the reported number of new cases of the chronic form of human West African trypanosomiasis (*T. b. gambiense*) fell by 97%, from 27 862 to 864. Importantly, the number of health facilities providing gambiense HAT diagnosis and treatment keeps increasing. Therefore, it can be considered that the observed trends are very likely to reflect a real abatement in disease transmission, despite the challenges always posed by under-detection. Notwithstanding the encouraging indicators, surveillance has weakened in South Sudan and the Central African Republic due to security constraints. So, the risk of deceleration is real and can have serious consequences as was already painfully experienced in the history of HAT.

*T. b. rhodesiense*: Uganda, Tanzania, Zambia, Malawi, Zimbabwe. No more cases reported from Burundi, Ethiopia, Kenya, Mozambique and Rwanda. This subspecies is a zoonosis affecting both wild animals and domestic cattle. Humans are sporadically infected “by accident”. Contrary to West African HAT, the zoonotic nature of rhodesiense HAT does not presently allow to envisage complete interruption of its transmission.

From 1999 till 2009, the number of newly reported cases of the acute form of human East African trypanosomiasis (*T. b. rhodesiense*) fell by 81% from 619 to 116. Of note, in 2018 only 24 cases of
East African HAT where reported. In Malawi the reported cases rose from 15 in 2018 to 91 in 2019. In contrast with the West African HAT, surveillance has weakened in countries as Tanzania, Uganda, Zambia and Zimbabwe. The replacement of microscopic examination for malaria diagnosis by rapid serological tests now prevents the accidental diagnosis of rhodesiense HAT when testing for malaria. This is exacerbated by a concomitant decrease in HAT-skilled staff who could maintain knowledge and awareness of the disease. Opposed to gambiense HAT, very few innovative tools have been developed for rhodesiense HAT screening, diagnosis and treatment. These factors in combination with the acute clinical progression of rhodesiense HAT usually prevalent in remote rural areas, are likely to result in non-negligible under-detection. An indirect indication of this under-detection is the fact that 8 cases (6% of the total rhodesiense HAT caseload) were diagnosed in non-endemic countries among returning tourists in 2015–2016.

Trypanosomiasis does occur in South America, but Chagas’ disease which is caused by *Trypanosoma cruzi* is clinically very different from African sleeping sickness. There are rare human infections with trypanosomes in India and Malaysia. They were due to accidental zoonotic infections with *Trypanosoma lewisi*, a rat and other rodents parasite transmitted by fleas, or *T. evansi*, a parasite mechanically transmitted by hematophagous biting flies and infecting mainly horses and camels but also buffalo and cattle. A number of human infections with *T. vivax* and *T. congolense* have also been reported. Such infections are very exceptional.

**Surra**

*Trypanosoma evansi* causes disease (“surra”) in certain animals, such as camels, llamas, horses, buffalo, cattle, dogs, sheep and goats. There is considerable variation in the pathogenicity of different strains and the susceptibility of different host species. The disease ranges from inducing a subclinical infection, mild disease, chronic to overt forms (months to years) and rapid fatal infections (esp. in horses and camels). Deer, capybara and coati can become infected and ill and may also constitute a reservoir. Animals subjected to stress such as malnutrition, pregnancy, work, are more susceptible to disease. Suramin is the most frequently used drug for treatment of surra in horses. Successful treatment by a single dose of diminazene diaceturate has been reported in dogs.

*Trypanosoma congolense* is the main trypanosome infecting cattle, causing animal African trypanosomiasis (AAT). Every year AAT is responsible for more than 3 million deaths in cattle with estimated annual agriculture economic losses of more than US$ 4.5 billion dollars, making AAT one of the major constrains for sustainable livestock production in Africa. A few indigenous African cow
breeds, such as the N’dama breed, tolerate the parasite’s presence remarkably well. However, these trypanotolerant animals are not popular with farmers because they grow slowly and are small. Many farmers prefer Boran cattle, which are more beefy with high resistance to heat and ticks but susceptible to AAT.

*Trypanosoma equiperdum* causes a chronic sexual transmitted disease (“dourine”) in horses, mules and donkeys. Infections are endemic in Eastern and Southern Africa, South America, Mongolia, Russia and Kyrgyzstan. *T. equiperdum* is the only trypanosome that is not transmitted by an insect vector.

**Parasite**

In general, among trypanosomes, one can distinguish several morphological forms based on the relative position of the kinetoplast to the nucleus. Extracellular African trypanosomes have two main morphologies:

1. Epimastigote: fusiform 20-40 µm long with an anterior placed kinetoplast, in front of the nucleus i.e. on the same side as the flagella is pointing. This stage occurs in the tsetse fly.

2. Trypomastigote: the kinetoplast is located behind the nucleus. The parasites are pleomorphic in human blood. Some are elongated and slender (“slender trypomastigotes”) and others are shorter and stumpy. Reproduction in man occurs via longitudinal binary cleavage every 7 hours.

In intracellular trypanosomes, like *T. cruzi* (see Chagas’ disease) the amastigote stage is present inside the cell. This multiplication stage is characterised by a spherical form without flagella.

**Parasite information**

The parasite has only one nucleus, is elongated, contains a giant mitochondrion and has a single flagellum. At the base of the flagellum is the basal body. This lies adjacent to the kinetoplast. The latter is a compact DNA (deoxyribonucleic acid) structure, located in the very long mitochondrion. This mitochondrion is almost as long as the entire trypanosome. The name of the Order to which the parasite belongs – Kinetoplastida – refers to this organelle. Between the basal body and the flagellum there is an undulating membrane which is required for motility. In the form of the parasite such as it occurs in man (trypomastigote), the kinetoplast lies in a posterior position and the flagellum points towards the front, rather like a bowsprit on a large sailing vessel. The parasite
occurs in the salivary glands of the tsetse fly as an epimastigote (kinetoplast located just in front of the nucleus).

The genome of *T. brucei* was sequenced and published in Science in July 2005. The DNA in the kinetoplast (kDNA) stains like that of the nucleus (recognizable on a smear). The structure of the DNA in this kinetoplast is very complex. There are numerous (about 40) large DNA loops (“maxicircles”) and even more (some 5,000-10,000) small DNA loops (“minicircles”).

While in the human host, the parasites are diploid. The parasites replicate in humans by asexual mitosis. Diploid and polyploid forms can be found in tsetse flies. Experimental arguments for meiosis and a possible sexual reproduction in *T. brucei* were first proposed in 1986. In the laboratory tsetse flies were infected with 2 different clones after which hybrid parasites were isolated, which indicates exchange of genetic material. This could be important for a better understanding of the natural parasite populations, e.g. via the various iso-enzyme patterns that occur in nature. Even if these laboratory data were confirmed, it remains an open question how important this is in nature.

The notion of **antigenic variation** in African trypanosomes has been around for a long time. Early investigators would isolate trypanosomes and serum from an animal early in the course of an infection and then again later during the same infection. Early antiserum would kill the initial strain of trypanosome, but did not affect the trypanosome strain isolated later in the infection. It was apparent that the trypanosome population changed over time. When the parasite is present in an individual it is covered with a thick monotonous layer of a single type of glycoprotein, VSG (Variant Surface Glycoprotein). The VSG coat is approximately 20% of total cell protein and includes more than 10 million molecules thus has a vast repertoire of surface antigens. The *T. brucei* genome has around 2000 distinct VSG genes but only one single VSG is expressed at a time. The entire VSG surface of a trypanosome is recycled every seven minutes by a process of VSG endocytosis and exocytosis. When the parasite is transferred to the tsetse fly, the VSG coating disappears within 4 hours and is replaced by an invariant glycoprotein (“procycline” or PARP (procyclic acidic repetitive protein)). After the parasite has completed its cycle in the fly, colonizes the salivary glands and transforms into the metacyclic infectious stage the VSG coating reappears. The metacyclic VSG coat is different from the bloodstream VSG coat having only 12 to 20 VSG types. The metacyclic VSG coat is supposed to limit the first immune response and thus facilitating the parasite establishment and proliferation in the vertebrate host, making the VSG coating of vital importance for the parasite. This explains why only metacyclic trypanosomes (the mature forms in the salivary glands of the insect) are infectious. When an antigenically homogeneous population of parasites is in the human body, antibodies against the
VSG of this population are produced. The immune system lyses the parasites which is accompanied by fever. Infections with trypanosomes would be cured quickly, if the parasite population could not constantly change its surface antigens. The switch of VSGs happens about once every 100 cell divisions.

Most of these VSG genes are located on specialized telomeric region defined as the expression site with around 80% of these telomeres residing on minichromosomes in the nucleus of the parasite. The parasite also has about twenty chromosomes of “normal” size. These do not condense during mitosis. At any one time, only one VSG gene per parasite is active. After destruction of the first dominant population by the immune system, the heterologous parasites increase in number until the variant VSG has induced antibodies and a new cycle of destruction begins. A third population of minority variants then emerges. Antigenic variation is a very important factor in the development of the disease and explains various symptoms (including its chronic course, fluctuating parasitemia and fever episodes).

**Vector**

Tsetse flies (*Glossina sp.*) are blood-sucking insects that occur only in sub-Saharan Africa and the Gisan oasis in Saudi Arabia. Four different species of fossil flies were discovered in 20-million-year-old mudrock in Colorado, USA, indicating that the insects once existed in North America. The name tsetse descends from the Tswana language. This name was also used by the Matabele and Zulus and refers to the sound that the insects make. An English reporter in Southern Africa at the end of the 19th century adopted this name when he wrote about a fly which attacked horses and cows.

The insects have prominent elongated mouthparts (proboscis), which explains their scientific generic name (“glossus”: tongue). Tsetse flies have typical wing veins, with a “hatchet cell” in the middle. When resting they fold their wings over their back like a closed pair of scissors. Other flies hold their wings more to the side. There are 31 species and subspecies, but less than half are vectors of human trypanosomiasis. The genus *Glossina* is now divided into three subgenera:

1. The fusca-group (subgenus Austenina): not important in human pathology.

2. The palpalis-group (subgenus Nemorhina): these flies prefer dense vegetation in humid areas (e.g. on riverbanks, gallery forests). Their habitat should have exactly the right conditions of humidity, warmth and light intensity, and there should be a blood supply (nearby animals or humans). Humans are frequently bitten when working/standing close to the water’s edge. The flies can also be found in cocoa, coffee, mango and banana plantations; this group is the vector of human West African
trypanosomiasis and nagana (= animal trypanosomiasis).

3. The morsitans-group (subgenus Glossina) are distributed over the East African savanna and are zoophilic. They are the vector of East African trypanosomiasis.

**Tsetse flies and their bloody bites**

As obligate blood feeders, both male and female tsetse flies feed with blood every 3 to 4 days. After landing on a host, the fly will lower its proboscis to a vertical position and stab with a rocking motion of the body. The rough dentate part of the proboscis saws through the tissues. The proboscis penetrates the skin while the teeth lacerate the capillaries walls and saliva is injected forming a small pool under the skin. The blood is actively pumped up and stored in the crop for a short time and is then passed to the midgut. Tsetse blood feeding implies manipulation of the host haemostasis, possible by producing and injecting a potent saliva anticoagulant cocktail at the biting site. Until now, two key molecules have been identified to facilitate tsetse blood feeding: the tsetse thrombin inhibitor with anticoagulant activities and the 5’Nuc apyrase with a dual role in platelet activation and aggregation. Bites of forest flies are less painful than those of savanna species. During a bite, tsetse can injects with the saliva infectious metacyclic trypanosomes. Feeding time ranges from 20 to 25 seconds. In a single meal 5-80 mg (max 155 mg) of blood in taken up. A hungry fly can take up a bloodmeal greater than its fasting weight. When satiated, the fly heads to a roosting site to digest at leisure.

Tsetse flies live a few months. If parasites are taken up by a bloodmeal, 99% of the parasites die in the insect’s stomach (midgut), but some transform in the procyclic (midgut) and later into the metacyclic trypomastigotes (salivary glands) The tsetse fly becomes infective 2 to 3 weeks after an infective bloodmeal.

Adult tsetse flies are airborne for short periods and rest for the remaining time. On average, they cover 200-300 meters in the dry season. In savanna areas they only take flight at times of the day when temperature is suitable. At the hottest time of the day (above 35°C) and during the night they rest. Farmers take advantage of this trait by driving their herds through infested areas after dusk. In forested areas where temperature swings are less marked they fly more often. There are several different species of tsetse flies, each with its own ecological preference. In an endemic area usually less than 1% of the flies are infected.

Congenital transmission is possible and there are case reports of laboratory accidents, blood
transfusion and organ transplantation as transmission route, but they are extremely rare.

**Clinical aspects**

**Infection by *T. b. gambiense***

Any bite from a tsetse fly, whether infected or not produces a local reaction. When the bite is infected a small local wound can appear after 1 or more weeks, but in general after 5-15 days (trypanosomal chancre or sore or trypanoma). This often remains unnoticed in the local population, though it can sometimes reach quite substantial dimensions (2-5 cm). In infected Europeans it is described at a frequency of 25-40%. It involves a central blister or ulcer surrounded by red infiltrated skin. The lesion tends to be minimal painful. When it has healed after 1-3 weeks a depigmented scar can remain. The infection develops slowly if there is no medical intervention. The patient’s condition gradually deteriorates, ultimately leading to his/her death in sometimes as short as a few months, sometimes much later. There are two quite artificially separated stages: a preliminary hematolymphatic stage and a second stage with symptoms of meningo-encephalitis. The boundary between these two stages is determined by the findings in the cerebrospinal fluid. The distinction is important for treatment. Asymptomatic human carriers (and spontaneous cure) are described but is rare.

**Hematolymphatic stage (first or early stage)**

The hematolymphatic stage lasts 6 to 12 months, but sometimes much longer. It is characterized by intermittent unpredictable bouts of fever separated by irregular intervals of days to a month or even more, headache and general malaise. The lymph nodes swell, especially those in the neck (Winterbottom sign). These glands are soft, mobile and not painful. In early West African trypanosomiasis, swollen posterior cervical lymph nodes are found in 50-85% of early stage patients and in fewer than 25% in the late stage. Oedema sometimes occurs (face), as well as pruritus (itching) and transient red spots or a circinate rash (trypanides). This rash can be seen without difficulty on a white skin (reported in 50%) but is difficult to see on a dark skin. The liver and certainly the spleen can be enlarged. There is moderate to severe anemia. Neurological disorders (personality changes), increased sensitivity to pain, especially deep hyperesthesia (“Kerandel sign”) can already be present in the first stage. This condition gradually evolves into increasing neurological collapse, characteristic of the meningo-encephalitic stage.

The condition is characterized by a chronic course with flare-ups and quieter periods. These flare-ups are to be interpreted as destruction of the trypanosomes, followed by the development of a new population of parasites carrying a different surface antigen. Lysis of the parasites releases large
quantities of antigen into the bloodstream. These form immune complexes with circulating antibodies which then precipitate resulting in perivascular inflammatory symptoms (including vasodilation with increased vascular permeability and oedema). Successive generations of parasites each have a different glycoprotein on the outer membrane. It is to this outer membrane that the antibodies attach themselves. Whenever a new glycoprotein emerges, the immune system always has to start again from scratch, with the production of new antibodies. This explains the pronounced increase in the immune globulins (especially IgM) in blood and cerebrospinal fluid. The high IgM serum concentration thus results from chronic polyclonal B cell stimulation. Aspecific cross-reacting and auto-antibodies can also be produced, making serological diagnosis of other diseases more difficult. Meanwhile time goes on and the infection worsens.

Meningo-encephalitic stage (second or late stage)

If left untreated, the meningo-encephalitic stage will progress to death in 6 months to 2 years after neurologic symptoms arise. Personality changes increase and the patient usually loses interest in their surroundings. Psychosis sometimes occurs. The patient develops tremor, paresthesia, increased sensitivity to pain, gait disorders, speech difficulties and reversal of the diurnal wake/sleep rhythm. Ataxic dyskinesia is present in most patients. Basal ganglia involvement can produce clinical features which overlap with those of Parkinson’s disease. Weight loss and endocrine abnormalities with e.g. impotence are common. Damage to the hypothalamus (paraventricular and supraoptic nuclei) may lead to disturbance of the normal sleep pattern. The patient progressively deteriorates and develops stupor (sleeping sickness!). The patient can still be woken up but will quickly go “back to sleep” again. Daytime sleeping, insomnia and behaviour change are reported in 40%, 55% and 30% of cases respectively. This is finally followed by coma and the patient dies of malnutrition, concomitant infections, accidents and destruction of the central nervous system. This disease is not to be confused with neurosyphilis, tuberculosis, AIDS with cerebral toxoplasmosis or cryptococcal meningitis, alcoholism or schizophrenia.

Histopathological changes include leukoencephalitis with demyelination and accentuation of the periventricular areas. There is a characteristic infiltration of lymphocytes and plasma cells around cerebral blood vessels (perivascular cuffing).

**Infection by *T. b. rhodesiense***

Infection with *T. b. rhodesiense* evolves much faster than West African trypanosomiasis. The incubation phase is shorter (1 to 3 weeks). An inoculation chancre often occurs (in traveller this is almost always present), and appears some days before the onset of pyrexia. There is high fever and
most patients have signs of multiorgan failure. Hepatitis leads to jaundice, elevated liver enzymes and coagulation disturbances. Myocarditis is common and often gives diffuse T-wave inversions. Heart failure can occur. ARDS can be detected on chest X-ray. Encephalitis leads to neurological symptoms, such as confusion and stupor. Daytime sleeping, insomnia and behaviour change are reported in 75%, 65% and 20% of cases respectively. There is usually no obvious lymph node swelling, but splenomegaly is frequent. The disease evolves to a fatal outcome within a few weeks or months.

**Diagnosis**

**Detection of parasites**

In the peripheral blood there is usually a normal white blood cell count (no leukocytosis or leukopenia), a normal platelet count and a slight normocytic anemia. The erythrocyte sedimentation rate is quite high, in part explained by the high immunoglobulins. The diagnosis is best made by detection of the parasite. The sensitivity of conventional parasitological techniques is however quite low. The parasite can be found in fluid from the inoculation chancre, blood (direct examination, thin smear, thick smear, buffy coat), lymph node fluid (needle aspiration) or cerebrospinal fluid (lumbar puncture). In a wet blood smear, the motility of the parasites attracts the eye but the sensitivity of the technique is too low. A Giemsa-stained thick blood smear is more sensitive, but parasites are frequently deformed in this preparation and are therefore easily missed. Lymph node aspiration is done with a dry needle. After puncture the needle is left in place for a while and the node is massaged. A syringe is then fitted to the needle and after aspiration the fluid is put on a microscope slide for direct examination (the motile trypanosomes can then be observed). Several samples will often be needed, as the parasites are not present in large numbers and appear in the blood in intermittent waves. Concentration techniques facilitate the diagnosis: Woo technique, buffy coat from a centrifuged microhematocrit tube or quantitative buffy coat test (QBC). In well-equipped laboratories a miniature anion exchange centrifugation technique (mAECT) is used (Lanham or Lumsden method). Such a column contains diethylaminoethyl-cellulose (DEAE-52). The separation of blood cells from trypanosomes depends on a difference in surface charge of the blood cells and the parasites. This charge is pH-dependent (importance of iso-electric point). Blood is mixed with a particular buffer (PSG = Phosphate-Saline-Glucose) and gently layered on top of the column. The blood will penetrate the gel on top of the column and red and white blood cells adhere to the DEAE gel particles. In this buffer, the trypanosomes are at their isoelectric point (=neither positive nor negative charge) so flow through the column. The eluate containing the parasites is collected and centrifuged. The sediment is examined microscopically to determine if parasites are present. The type of buffer and the temperature at which the test is carried out are of very great importance. The more the disease advances the less frequently are trypanosomes found in the blood, though they are then
found more often in the cerebrospinal fluid. The parasites can be cultured in vitro in a specific medium (KIVI; Kit for in Vitro Isolation). In theory as few as 1 trypanosome can be detected in 5 ml, though in 50% of the tested cases the culture remains sterile.

**Comparison of detection thresholds**

- Fresh blood preparation (10 µl) 6000 trypanosomes/ml
- Thick drop (10 µl) 2000 trypanosomes/ml
- Buffy coat (70 µl) 600 trypanosomes/ml
- QBC (Quantitative Buffy Coat Test) 16 trypanosomes/ml
- MAECT (500 µl) usually 100/ml required
- PCR (Polymerase Chain Reaction) 10 trypanosomes/ml
- KIVI (Kit for In Vitro Isolation) 1 trypanosome per 5 ml.

**Serology**

Antibodies can be detected serologically. Several techniques (immunofluorescence etc.) have been developed. There are also methods for use in primitive rural conditions. A cheap and practical method is a direct agglutination reaction of trypanosomes on a plastic card, with macroscopic read-off (**CATT = Card Agglutination Test for Trypanosomiasis**), which was developed by the Institute of Tropical Medicine, Antwerp. This is a good screening method for *T. b. gambiense* in most areas. The sensitivity of the CATT test in areas (e.g. Cameroon) with *T. gambiense* strains which do not carry the variable surface antigen LiTat 1.3 is lower. A drop of blood (finger prick) and a drop of reagent that contains blue-colored parasites of a known serotype are mixed on a white plastic card. The card is mechanically shaken for 5 minutes and then immediately read. When the test is positive (presence of antibodies) the trypanosomes agglutinate and form a blue clot. The CATT has no place in the diagnosis of *T. rhodesiense* infections, except in a chronic form of *T. rhodesiense* which exist in Malawi. CATT must not be confused with the CIATT (Card Indirect Agglutination Test for Trypanosomiasis, an antigen-detection test). Another method is to take a blood drop on very small filter papers (confetti) and examine this later in a laboratory. The patient should be called back later if the result is positive. Antigen-detection methods (ELISA) have also been developed, but are not yet in
routine use. A problem arises in persons who have a positive serology, but who are asymptomatic and in whom no parasites are found (wait and see with follow-up or treatment with suramin or pentamidine?). After successful treatment the antibodies remain for years. Antibody detection therefore cannot be used for detecting relapse or reinfection. It is hoped that in the future we shall be able to prove a cure by monitoring reductions in the levels of circulating antigens.

The CATT is designed for mass screening and still requires agitator rotator, electricity, and refrigeration, there is a need for simple and individual point-of-care tests. In 2013, two lateral-flow rapid diagnostic antibody-detection tests were developed for *T. brucei gambiense*: the HAT Sero-K-SeT test (Coris, Belgium) and the immunochromatographic HAT-RDT (Standard Diagnostics, Korea), designed for testing on whole blood, with results provided within 15 minutes. The tests contain variant surface glycoproteins (LiTat 1.3 and LiTat 1.5). Clinical field evaluation showed sensitivity and specificity similar to those obtained with the CATT, but with simpler use (no need of electricity and cold chain). In large multicenter prospective studies, sensitivity of both SD HAT-RDTs was found lower than expected (71%–89%) whereas specificity was very high (98%). However, combining any of these RDTs together or with CATT achieved a very high sensitivity. It appears therefore that both these RDTs achieve a diagnostic accuracy equivalent to that of CATT and may be used instead for both mass screening and clinical care, wherever local conditions do not favour the use of CATT.

In *Trypanosoma b. r.* HAT, the parasite load in blood is usually very high at symptom onset, so trypanosomes are relatively easily detected whenever a blood smear is performed. In travellers, diagnosis of *T. brucei rhodesiense* HAT has been almost always made by thick and thin blood film examination, often as a surprise finding. It could, however, be missed when in such circumstances malaria diagnosis is limited to RDT. Although antibody based assays exist for *T. brucei rhodesiense* HAT, none have been developed in RDT format.

### Genomic tests

The first PCR was developed in 1983 by Kary Mullis (Nobel Prize Chemistry 1993). Since then several PCR variants have been developed. Most techniques consist of an amplification step followed by amplicon electrophoresis in agarose gel, but there are other approaches. The sensitivity and specificity largely depend on the DNA sequence targeted by the primers. Preferred genomic sequences are those which are conserved and unique for the parasite and that occur as multiple copies in the genome. Tests based on extra-nuclear minicircle kinetoplast DNA have failed to live up to expectations. With PCR, formal molecular differentiation between *T. brucei gambiense*
and *T. brucei rhodesiense* is possible. *T. brucei gambiense*-specific glycoprotein is only present in *T. brucei gambiense*, while the gene encoding the serum-resistance-associated protein (SRA) is only present in *T. brucei rhodesiense*. Both however are single copy genes. The most interesting next-generation diagnostic for active infection by trypanosomatids is the spliced leader RNA (SL-RNA) detected by PCR. The splice leader is a conserved species specific sequence capping the mature mRNAs.

**Clinical diagnosis**

A correct diagnosis can sometimes be reached even though parasites cannot be detected. These “clinical cases” are patients from an endemic area, with clinical symptoms of late stage trypanosomiasis and lymphocytes in the cerebrospinal fluid. Such “clinical cases” may amount to no more than 5% of the total number of trypanosomiasis patients.

**Diagnosis, IgM in cerebrospinal fluid**

Antibodies should if possible be detected in the cerebrospinal fluid. Determining the IgM content in the cerebrospinal fluid can be very difficult or even impossible to carry out in endemic areas and under field conditions. An experimental latex agglutination test for detection of IgM was developed at the Institute of Tropical Medicine, Antwerp, Belgium. Blood-CSF barrier dysfunction is usually absent or mild and occurs in very advanced late-stage disease. It is possible to calculate and plot diagrams of the quotients CSF/serum concentration for IgG, IgA and IgM (demonstration of intrathecal production of antibodies). Especially intrathecal IgM production will be present in late-stage sleeping disease (occurs in 98% of people with leukocyte counts higher than 20/µl). Similar patterns do occasionally occur in Lyme neuroborreliosis, neurosyphilis, mumps meningoencephalitis and in non-Hodgkin lymphoma involving the central nervous system.

**Usefulness of the lumbar puncture**

A lumbar puncture is important:

1. sometimes in order to make a diagnosis
2. in order to determine the stage (main purpose)
3. in order to monitor therapy
In the 2nd stage the cerebrospinal fluid is characterized by:

- white blood cell count (WBC) > 5 per mm³, (normally <3)
- protein > 45 mg% (normally 15-45 mg%)
- IgM increase (difficult to carry out; Latex IgM)
- sometimes trypanosomes and/or Mott cells (= degenerated plasma cells: multiple varied size spherical inclusions/ Russell bodies within a plasma cell having an eccentrically placed clock face nucleus; also called morula cells of Mott (in Latin ‘morus’ means mulberry).

**Treatment**

There are several different treatment schemes that are determined by the vertical control program that is (or was) in place in many areas. The specific therapy is not simple. Drugs that do not penetrate into the cerebrospinal fluid and the brain are useful in the early stage only (prior to invasion of the central nervous system). Drugs that do penetrate the blood-brain barrier must be used in the late stage. Although recent progress has been made, there is an urgent need for less toxic, easy to administrate and cheaper drugs.

Here under are summarized the current guidelines for HAT treatment, according to the causal species and disease stage. Thereafter, each trypanocidal drug is described one by one for information.

**Treatment summary**

**T. b. gambiense early stage:**

Pentamidine 4 mg /kg/day IM or IV for 7 days (preferred)

Or suramin test dose 5 mg/kg, then 20 mg/kg/day (max. 1 g) IV on days 3, 10, 17, 24, 31 (alternative)

**T. b. gambiense late stage:**

NECT: eflornithine 200 mg/kg IV 2 times per day for 7 days + nifurtimox 5 mg/kg/day TID orally for 10 days

Or eflornithine 100 mg/kg IV 4 times per day for 14 days

NB: Melarsoprolol is not used any more for *T.b. gambiense* except in the very rare situations of
treatment failure with NECT, and after expert advice. The regimen is then similar to the treatment of rhodesiense HAT late stage (see below: 2.2 mg/kg/day for 10 days with prednisolone throughout the whole period)

**T. b. rhodesiense early stage:**

Suramin test dose of 100 mg (check urine for protein and cylinders), then 20 mg/kg/day (max 1 g) on day 1, 3, 7, 14 and 21 (alternative 20 mg/kg weekly for 5 weeks)

**T. b. rhodesiense late stage:**

Melarsoprol 2.2 mg/kg/day for 10 days under cover of prednisolone (see above); the previous cumbersome alternative (3-4 series of 3.6 mg/kg/day IV for 3 days weekly) is being abandoned

NB: The first all-oral short course treatment fexinidazole has been approved in 2020 by WHO as a valid alternative treatment for *T.b. gambiense* early stage AND late stage (but only in children 6 years or more, if drug administration with food is directly observed T, and for the late stage if there is no advanced neurological disease nor presence of more than 100 WBC/µL in CSF examination). Details about the rationale are provided below in the paragraph on fexinidazole.

**Trypanocidal drugs**

**Suramin** (Germanine®). The compound was developed in 1920. It is best administered by slow intravenous infusion, as intramuscular administration (10% solution in distilled water) is very painful. The drug is active against both *T.b. gambiense* and *rhodesiense*, but its toxicity restricts its use to the latter pathogen, since a better tolerated alternative (pentamidine) exists for the former. Suramin is excreted extremely slowly by the body. This is important if exfoliative dermatitis develops as a side effect. It can cause substantial proteinuria and a nephrotic syndrome. When a test dose of 100 mg is tolerated well, the daily dose 20 mg/kg (max. 1 g) can be given on days 1, 3, 7, 14 and 21. A urine strip should be performed before each administration, to look for occurrence of proteinuria. In the past 20 mg/kg (max 1g) weekly was given for 5 weeks. Fever sometimes initially occurs due to lysis of trypanosomes. Suramin also kills *Onchocerca volvulus* filaria. Patients with active onchocerciasis can exhibit severe side effects to suramin (cfr. Mazzotti reaction with DEC).

**Pentamidine** was developed in 1941. It is less active than suramin and not active against *T. b. rhodesiense*. pentamidine exists as an isethionate salt (Pentacarinat®, Pentam®) and must be administered parenterally. Intramuscular injections are painful and therefor slow IV administration is
preferred. Rapid IV injection causes acute hypotension. Hypoglycemia can sometimes occur due to release of insulin from the pancreas. Other adverse events include pancreatitis, ventricular arrhythmias, hepatotoxicity and kidney failure. This medicine is also used in pneumocystosis in AIDS patients and the treatment of cutaneous *Leishmania guyanensis*. The recommended field treatment is 4 mg kg/day for one week.

**Eflornithine** (DFMO, Ornidyl®). Di-fluoro-methyl-ornithine or DFMO was first used for trypanosomiasis in 1985. It is very water soluble. This substance penetrates quite well into the cerebrospinal fluid. A cumbersome IV treatment is however required, divided in 4 administrations per day for 2 weeks. Eflornithine is rather well tolerated, although hematotoxicity is possible (and bacterial infection of IV lines in the tropics) as well as seizures. Unfortunately it is active only against *T. b. gambiense*. In monotherapy, the dosage regimen is 100 mg/kg/6 hours IV x 2 weeks via physiological fluid infusion. Concentrations in cerebrospinal fluid in children seem to be lower than in adults. Children require a higher dose (150 mg/kg 4 times a day). If used in combination with nifurtimox, the dose is 200 mg/kg 2 times a day (over 1 hour) for 7 days.

**Nifurtimox** (Lampit®): Cf. Chagas’ disease. After thorough pharmacokinetic studies, a multicenter trial has evaluated the safety and efficacy of the combination of nifurtimox (oral, for 10 days) with eflornithine (IV, 2 administrations of 200/kg per day for 7 days), compared to eflornithine in monotherapy (100 mg/kg 4 times a day) for 14 days. The nifurtimox-eflornithine combination treatment (NECT) was not inferior to eflornithine (> 95% cure rate) but much easier to administer and cheaper (14 infusions of eflornithine instead of 56!). Further field phase 4 studies confirmed these excellent results. This combination has also the theoretical advantage to prevent emergence of resistance. The NECT has been endorsed by WHO in 2012 and has become the first-line therapy in all endemic countries. The drugs are provided for free by WHO to the national HAT programs.

**Melarsoprol** (Arsobal®) was developed in 1949. Because of the demonstrated efficacy of eflornithine for second stage *T. b. gambiense* trypanosomiasis (with limited side effects), the use of melarsoprol is nowadays limited to the *T. b. rhodesiense* form (second stage). This trivalent arsenic compound is insoluble in water or alcohol. It is therefore dissolved in propyleneglycol. This solvent is highly irritant to tissues. It causes phlebitis and chemical cellulitis when administered paravenously. Melarsoprol may only be given by very slow IV infusion. It has a significant trypanocidal activity (as can be measured via bioassay) in plasma and cerebrospinal fluid for up to several days after administration, although melarsoprol can then no longer be detected with HPLC (high performance liquid chromatography). The molecule is transformed into biologically active metabolites such as melarsene oxide that irreversibly binds to pyruvate kinase, which disrupts energy production in the parasite. The same effects happen in host cells, rendering the drug highly toxic. Resistance to melarsoprol is
described. Toxicity results in polyneuropathy and reactive encephalitis. Encephalopathy tends to manifest itself as a sudden violent neurological deterioration at the end of the first series or during the second series of injections. At present, there seems to be no way to predict which patient will develop encephalopathy. Corticosteroids seem to diminish the risk and severity of the encephalopathy (controversial). It is therefore imperative to administer prednisolone before using melarsoprol. In general, one can expect lethal reactive encephalopathy after administration of melarsoprol in about 3-5% of cases. Clinically, there are three syndromes of reactive arsenic encephalopathy:

- convulsive status associated with acute cerebral oedema, due to diffuse lesions with hemorrhagic encephalitis.
- rapid progressive coma without convulsions
- acute nonlethal mental disturbances without neurological signs (e.g. psychosis)

Another toxic effect of melarsoprol is polyneuropathy (analogous to heavy metal intoxication). This results in diminished sensitivity and/or paresthesia’s in hands and feet, as well as motor signs. In this case melarsoprol should if possible be stopped and vitamin B (e.g. thiamine) administered.

In the past, treatment regimens consisted of 12 injections over a 30-day period, but current regimens with daily administration of 2.2 mg/kg for 10 consecutive days have proven a similar efficacy towards *T. b. rhodesiense* and *gambiense* without increase in toxicity, avoiding very long hospitalizations.

**Experimental medications**

Fexinidazole is a product related to megazol, tinidazole and metrinidazole. After demonstrations of in vivo trypanicidal activity in 1983 the drug languished in obscurity for more than 25 years. Fexinidazole was “rediscovered” as an oral drug which might be used in early and late stage sleeping disease. In African trypanosomes, the mode of action of nitro drugs involves reductive activation via a NADH (reduced form of nicotinamide adenine dinucleotide)-dependent bacterial-like nitro-reductase. In a randomized controlled trial including 394 patients, treatment success at 18 months was slightly lower with fexinidazole (1800 mg once a day, days 1 to 4; 1200 mg/day, days 5 to 10) than with NECT therapy (91% vs 98%) but this difference was within the predetermined acceptability margin of 13%. The death rates (none directly attributable to treatment) and severe adverse events were similar between the two groups. In a subgroup of patients with > 100 >WBC/µl in the CSF, fexinidazole was inferior to NECT (87% vs 99% cure). Based on these new key findings, the WHO has revised its recommendations in 2020, and considers nowadays fexinidazole as a valid treatment for BOTH stage 1 and stage 2 gambiense HAT, PROVIDED that the patient does not present with advanced
neurological disease (and/or > 100 WBC/µL in CSF examination) and that treatment administration can be directly observed (lower efficacy if no concomitant food intake). Implementation of this new strategy is just starting.

In sharp contrast with the gambiense HAT, rhodesiense HAT treatment did not progress at all. Studies to examine the efficacy of fexinidazole against *T. b. rhodesiense* are ongoing. If successful, fexinidazole has the potential to become a safe, efficacious, affordable, oral short-course for both stages of *T. b. gambiense* and *rhodesiense*.

The Drug for Neglected Diseases Initiative (DNDI) has recently developed the compound SCYX-7158, the first oxaborole-based agent (the boron atom being essential for the trypanocidal activity even if the mechanism of action is unknown). Phase 1 studies have been completed. Phase 2/3 study results are expected in 2021. The drug has a very long half-life (17 days in healthy volunteers), making it very promising as a single dose oral treatment for both HAT studies.

**Treatment follow-up**

After treatment the patient should have a regular follow-up for 2 to 3 years for possible relapse (*T. b. gambiense*: check every 6 months). The first sign of relapse is often an increase in the cell count in the cerebrospinal fluid, followed by a rise in its protein content. Recurring fever, drowsiness and chronic headache are also signs of relapse. Unfortunately, obtaining a CATT negative result after treatment for HAT cannot be relied upon to confirm successful treatment. Further works needs to be done to address this question.

Recent studies have however demonstrated that it was possible to simplify and shorten the period of follow up after treatment for second stage *T. b. gambiense* infection according the following rules:

- At 6 months presence of trypanosomes or WBC > 50 in the CSF are considered as failures; WBC count < 5 in the CSF is considered as a cure (no further lumbar puncture). The remaining patients need to be re-evaluated again at 12 months by lumbar puncture.
- At 12 months: cure if no trypanosomes and CSF WBC < 20; failure if > 20 WBC in CSF or trypanosome.
American trypanosomiasis (Chagas’ disease)

Summary

- *Trypanosoma cruzi*, only in the New World
- Transmission via bugs, blood transfusion, congenitally and orally (bug feces in food/drink)
- Importance of poverty (housing) in transmission
- Acute (especially children): chancre, Romaña’s sign, fever, lymphadenophathy, myocarditis, hepatosplenomegaly
- Chronic: cardiac arrhythmias, heart failure, emboli, apical aneurysms
- Chronic: dysphagia, constipation (mega-syndrome)
- Diagnosis: clinical + thick smear/buffy coat (early), serology, xenodiagnosis, ECG, X-ray (late), PCR
- Treatment in the early phase still reasonably successful with medication; in the late phase difficult and probably useless
- Nifurtimox badly tolerated as a 2 to 4-month treatment; benznidazole: problems with bone marrow toxicity, hypersensitivity, peripheral neuropathy.
- Prevention: much progress in recent years via vector control and control of blood banks.

Introduction

**Historical note**

In 1907 the physician Carlos Chagas (1879-1934) was working in Lassance, a small poverty-stricken town on the Sao Francisco river in the state of Minas Gerais, Brazil. The town had been built along the railway from Rio de Janeiro to Belem. Chagas treated the workmen for injuries, syphilis, malaria etc. He noticed that cardiac arrhythmias occurred frequently. One day an engineer brought him an insect of the type which was known to often suck the blood of humans at night. Chagas wondered if this creature could also transmit malaria like the Anopheles mosquitoes. In the bug he discovered a unicellular parasite. In April 1908 he found the same parasite in a sick cat. Two weeks later, in the same house, the parasite was found in the blood of a 3-year-old child (Rita), who was ill with fever. Her face, liver, spleen and lymph nodes were swollen and the child died shortly afterwards. In the house there were countless bugs which tested positive for the parasite. He sent bugs to Rio, to Oswaldo Cruz, his former teacher (Brazilian physician 1872-1917). In the laboratory the parasite caused an infection in marmoset monkeys (Callithrix sp.), rodents and puppies. The disease caused by this parasite, American trypanosomiasis, was named after Chagas. The parasite was given the name Trypanosoma cruzi. The parasite did not always trigger
disease, however. In 1908 Chagas also discovered the parasite in another person (Bernice). This woman died in 1989, still infected, but without signs of organ involvement.

The infection apparently already existed before contact with the West. In 1985, 22 mummies were found in the Andes mountains. These were 1500 years old. In approximately half of them the heart, colon and/or oesophagus were clearly enlarged (lesions typical for Chagas’ disease). *Trypanosoma cruzi* DNA was found in 1999 in a 4000 year-old mummy in Northern Chile. In one of his books Charles Darwin describes how in 1835 in South America he was bitten by the bugs. It is possible that he incurred infection and later developed a chronic form of the disease.

**Distribution**

The infection only occurs in America in endemic regions. It is a disease associated directly with poverty. The severity varies from region to region. In the South of Texas there are very few cases. Infections occur in Central America sporadically. Although the disease is endemic in large areas of South America (in particular in the “Gran Chaco” region), the majority of those infected have no symptoms. Until recently it was thought that approximately 16 million persons were infected, but these figures are under review (see Prevention). The disease is transmitted via the faeces of an infected bug.

**Reservoir**

The parasite, *Trypanosoma cruzi*, occurs in more than 100 species of mammal (opossums, guinea pigs, goats, dogs, cats, rats, mice, and so on). There are several known (and probably also some unknown) subtypes each of which has its own distribution and probably also its own pathogenic features. In view of the extent of the animal reservoir eradication of the parasite will not be possible. This does not mean that the disease and the transmission cannot themselves be controlled. At present the strains are divided into two groups. *Trypanosoma cruzi* I has an extensive sylvatic reservoir, of which opossums appear the most important. This group is not very common in the “Southern Cone” countries (Argentina, Brazil, Chile, Paraguay, Uruguay), but it is virtually the only form which occurs north of the Amazon region. T. cruzi II seems to be chiefly associated with rodents and is common in the Southern Cone.

**Transmission**

Transmission occurs chiefly via infected bugs. These large insects like to bite sleeping humans at night (a mosquito net gives protection). They have a sharp proboscis which at rest is folded below the head like a jack-knife. When biting they inject anticoagulants and an anesthetic substance into the
wound. Since this makes their bite quite painless (kissing bugs), people seldom wake up and several bites may take place unnoticed in the course of one night. The parasite is not inoculated directly by the bite, as Chagas initially thought. In 1913 Brumpt showed that the parasite is found in the faeces of the insect. While the animals suck blood, they defecate. By scratching, a bitten person can bring the faeces into the bite wound or rub them into the conjunctiva. The parasites multiply in humans and then appear in the blood. The cycle is completed when a subsequent bug drinks infected blood. In the bug the parasite undergoes further changes and after 2 to 3 weeks is excreted with the faeces during a subsequent bite. It is estimated that the risk per bite by an infected *Triatoma* is one in a thousand. The existence of **oral transmission** has been suspected for quite a while. It was demonstrated in animals and has now been confirmed in some human cases. How frequent oral transmission happens is not clear yet. Food or drink contaminated with the liquid faeces of infected bugs or containing (crushed) dead bugs may lead to infection not only in experimental animals but also in humans. Small outbreaks of acute Chagas are regularly reported from Northern Brazil in the last years. The parasite could withstand short periods of freezing, but not decontamination with sodium hypochlorite or heating to 80°C. **Congenital infection** (1 to 2 % risk) and transmission via **blood transfusion** also occur (poor people often sell their blood). To give an idea of the scale, this implies for example that several thousands of babies are born with congenital Chagas each year in the USA, and a lesser number in Europe (from immigrant mothers from endemic areas). Transmission via transfusion is particularly important in urban zones and has been reported outside endemic countries. The risk of infection after a contaminated blood transfusion is estimated at one in five. There are sporadic cases of **accidental contamination** of laboratory staff (finger prick, aerosol) and after **organ transplantation** (including in non-endemic countries).

**Vectors**

The bugs are also known locally as “vinchucas” or “barbeiros”. Of the approximately 120 vector species only about 7 are important. Each species has its own region of distribution:

- Central America and northern South America: *Triatoma dimidiata* and *Rhodnius prolixus*
- South America (south of 5° S): *T. infestans*, *T. braziliensis*, *T. sordida*, *Panstrongylus megistus*

The bugs mentioned here are the main vectors. Other bugs also play a part in different regions. The bugs each have their own preferred biotopes. *T. dimidiata*, for example, is often found inside houses on the floor or the lower 150 cm of the walls or immediately outside in dung heaps, hollow trees, etc. In contrast, *R. prolixus* prefers to live in palm leaves either in the roof of the house or in the tree itself. In and around the house the bugs can feed on animals (e.g. dogs are important because they sleep at night when the bugs are active). The vectors often live in chicken runs but the chickens themselves
are not infected (they do eat bugs). During the day the insects hide in all kinds of cracks and crannies (importance of earthen or adobe walls) and in the roofing (straw, wood, etc). It can be seen immediately that the key word in Chagas’ disease is “poverty”. These are insects which reproduce slowly and whose geographical spread is slow. Migration of bugs, by migrating birds for example still needs to be studied. In view of these characteristics and the fact that the important vectors live around houses they can easily be reached by eradication campaigns.

A fertilized female lays several hundred eggs in her lifetime. From the egg comes a nymph which always needs a blood meal for its subsequent development stages (both sexes suck blood). The last instar will develop into an adult insect. During a blood meal they suck more than their own weight in blood. This takes 10-25 minutes. The insects may live for up to 2 years (5 years for T. barberi). Rhodnius prolixus has a relatively short generation time (3-5 months), while for T. dimidiata this time is quite long (1 year or longer). Long generation times make the development of resistance to insecticides difficult.

Parasite

In stained blood preparations the parasites are C- or S-shaped with a prominent kinetoplast towards the rear (trypomastigotes). The nucleus is elongated and the undulating membrane is usually not clearly visible. After infection multiplication of the parasite in the human is solely intracellular. They form microscopic pseudocysts in the tissues (similar to toxoplasmosis and sarcocystosis). This occurs mainly in the heart, muscle cells, some nerve cells and the lymphatic system. In the cell the parasite is small and rounded with no flagellum (amastigote). When the infected cell ruptures, parasites are released into the blood circulation where they become elongated and develop a flagellum. These forms can then infect other cells or be ingested by a bug.

Clinical aspects

Infection and incubation

Incubation period after exposure to vector-borne T. cruzi is 1 to 2 weeks, although longer incubation times are sometimes reported. If the parasites penetrate via the conjunctiva, there is unilateral redness and oedema of the upper and lower eyelids after 4 to 12 days. This is “Romaña’s sign”, named after the Argentinean physician Cecilio Romaña, who described the oedema in 1935. This swelling may last for weeks. Sometimes there is also swelling of the ipsilateral lymph nodes (including the pre-auricular lymph nodes). Trypanosomes may be found in the tears at this stage. If inoculation is in the skin there is local oedema and redness (“chagoma”) in 75% of cases. This remains for 1 to 4
months. From these sites the infections spreads.

**Acute stage**

The incubation period is followed by the acute phase which lasts 4 to 8 weeks. Many infections are initially asymptomatic. Acute symptoms occur more frequently in children than in adults. Dissemination of the parasite from the inoculation site may go unnoticed but may also give rise to acute illness with muscle pain, local or generalized oedema, swollen liver, spleen and lymph nodes. Moderate fever is almost always present in symptomatic cases and may persist for a long time, two or even four months. Sometimes there is also acute inflammation of the heart (myocarditis) with arrhythmias, decreased blood pressure, and heart failure. As with other forms of myocarditis the electrocardiogram is frequently abnormal. There is low QRS-voltage, prolonged PR- and/or QT-interval, T-wave abnormalities. Rarely there are ventricular extrasystoles or atrial fibrillation (the prognosis is poor if this occurs). Acute inflammation of the brain and meninges (meningo-encephalitis) occurs, chiefly in young children. Inflammation of the heart and brain may be fatal. There is pronounced lymphocytosis and monocytosis. The acute-phase case fatality rate is nowadays estimated to be 0.25 to 0.50% with early treatment.

**Latent period**

If the patient survives the initial phase (which is usually the case), a latent period occurs of indeterminate duration. The patient is asymptomatic, seropositive and the parasitemia is very low. Focal lesions are found in 60% of endomyocardial biopsies from patients in the latent phase. A positive xenodiagnosis can be obtained in 50% to 100% of these patients. For xenodiagnosis 10 to 40 non-infected bugs (e.g. *Dipetalogaster maxima* or *Triatoma infestans*) feed on blood from the patient. The faeces from these animals are investigated after 30, 60 and 90 days. In the event of immunosuppression there may be an acute flare-up, including meningo-encephalitis associated with AIDS or heart transplantation.

**Chronic phase**

Gradually the patient develops symptoms. These vary greatly from region to region. Lesions of the heart, oesophagus and colon are the most common.

Chronic heart problems
Chronic damage to the heart muscle cells and the cardiac conduction system (including that caused by auto-immune mechanisms) leads to heart failure. There is dyspnoea during exertion, orthopnoea and sometimes paroxysmal nightly dyspnoea, oedema of the feet and ankles, congestion of the neck veins, enlarged liver and crackles over the base of the lungs. Sometimes there is pulsus alternans: the peripheral arterial pulsations are alternately strong and weak. The precise pathophysiological mechanism is not fully known. The apex of the heart, which is normally situated on the mid-clavicular line, is displaced to the left. The heart sometimes becomes enormous, which may lead to clot formation in the heart. If blood clots break loose, there may be embolic complications: cerebrovascular accident (CVA), ischemia of limbs, renal infarction. Apical lesions in the left ventricle (wall thinning, intramural bleeding, aneurysms) are typical and occur in approximately 50% of patients. Unlike arteriosclerotic post-infarction aneurysms, in Chagas’ disease the apical cardiac tissue does not consist of scar tissue, the wall is simply thinned. Right ventricular lesions occur in 10 to 20%. Cardiac arrhythmias may cause palpitations, dizziness, syncope and sudden death. On the electrocardiogram a right bundle branch block is often seen, together with a left anterior hemiblock, ventricular extrasystoles, abnormal Q-waves and/or AV-conduction disturbances. The coronary arteries are normal. A complete left bundle branch block is exceptional, unlike in idiopathic dilated cardiomyopathy. Sudden death is common in people with Chagas’ disease. Probably this is due to ventricular tachycardia which changes suddenly into ventricular fibrillation.

In advanced heart failure, typical radiographic signs may be observed on a chest X-ray: cardiomegaly, prominent hili and distended pulmonary veins in the upper fields, pleural fluid, interstitial pulmonary oedema (fluid in the interlobular septa with Kerley B lines), peribronchial cuffing and finally alveolar pulmonary oedema (“butterfly oedema”).

The degree of heart failure is often indicated using the New York Heart Association classification:

- Grade I : asymptomatic
- Grade II : symptoms only during moderate to severe exertion
- Grade III : symptoms during mild exertion
- Grade IV : symptoms at rest. Patient generally confined to bed/chair.

Oesophagus and colon problems

Due to involvement of the small nerves in the oesophagus and colon, peristalsis is reduced and these organs are distended. This occurs in 5 to 10% of seropositive people south of the Amazon, and is virtually absent further north. *Trypanosoma cruzi* I and II are both associated with cardiac lesions, but intestinal lesions only occur in infection with *T. cruzi* II (the southern area).
Mega-oesophagus is characterized by difficulty in swallowing (dysphagia), choking, hiccups and nocturnal cough. This often leads to under-nourishment and loss of weight. Aspiration pneumonia is the most feared complication with substantial mortality. A clinical aid for detecting delayed oesophageal emptying is to measure the time between swallowing a mouthful of water, and observing the abdominal noises (stethoscope on the epigastrium). Normally this is less than 10 seconds. A distended oesophagus may also be shown on X-ray. The parotid gland may hypertrophy and lead to so-called “cat’s face”.

Mega-colon can lead to pronounced constipation, meteorism (abdominal distension), abdominal pain and functional intestinal obstruction due to involvement of the myenteric (Auerbach) plexus and the submucosal (Meissner's) plexus. The abdomen is often distended. Fecaloma, volvulus and peritonitis are complications.

The nervous system

In no other infectious disease is the involvement of the autonomous nervous system as important as in Chagas’ disease. Denervation of the parasympathetic nervous system is better documented and is much more pronounced than denervation of the sympathetic system. There can be sensorimotor polyneuritis. There is some hypoesthesia and paraesthesia, but chiefly a reduction or loss of tendon reflexes. The EMG is disturbed. In the central nervous system there is meningo-encephalitis in the acute phase, but the abnormalities in the chronic phase need to be better defined. In flare-up (e.g. AIDS) there may be intracranial hypertension, lesions of the cerebral nerves, paresis, plegia, stupor and convulsions. The cerebrospinal fluid exhibits a normal number of cells or pleocytosis with predominant lymphocytes and an elevated protein content. At times T. cruzi may even be detected in the cerebrospinal fluid. A CT scan of the brain shows one or more necrotizing lesions which may or may not be ring-shaped, with haemorrhages usually subcortical in the brain hemispheres and occasionally in the cerebellum or the brain stem. T. cruzi lesions rarely occur in the basal nuclei. These clinical pictures should be differentiated from cerebral toxoplasmosis, abscesses, mycoses, tuberculomata or other mycobacterial lesions, metastases or lymphoma.

Of all cerebral vascular accidents leading to stroke, about 20% are secondary to embolism from a blood clot secondary to atrial fibrillation. If patients do not take oral anticoagulants, an average of 5% CVA’s per year can be expected, which roughly translates to 50% of patients with CVA within 10 years after onset of atrial fibrillation. However for several reasons (mostly haemorrhagic) 20-40% of patients cannot be treated with oral anticoagulants. Most of the clots (90%) originate when blood stagnates in the left atrial appendage, also known as the left atrial auriculum.
**Congenital infection**

About 1 to 2% of babies born to seropositive mothers are infected. They may be asymptomatic (rarely) or may develop hepatosplenomegaly, neurological involvement, myocarditis, oedema and a bleeding tendency. The babies may be dysmature and/or premature. Fever is rare in these children. The mortality may be as high as 50% and they tend to die within a week. Those who survive will generally have permanent residual neurological damage.

**Diagnosis**

In the acute stage the parasite may be found in the blood via a thin blood smear, thick smear or buffy coat. As a concentration technique an anion-exchange minicolumn may be used (Woo’s technique similar to Lanham’s column, but with a different buffer, see African sleeping sickness). Strout’s concentration technique includes the double centrifugation of serum (from 10-20 ml of blood), after which the motile trypanosomes can be detected in the sediment. **PCR techniques** for *T. cruzi* exist, but can only be carried out in better equipped laboratories. The **serology** is positive from the fourth week. To know whether the neonate from a seropositive mother is infected, PCR is performed and IgM antibodies in its blood are determined. A positive serology (IgG) 6 months after birth also indicates infection. In-vitro and in-vivo culture is possible, but usually not available. Biopsies of lymph nodes, heart and muscles sometimes show parasitic pseudocysts (amastigotes in the cells). This is quite an aggressive technique and not very sensitive. *Dipetalogaster maximus* is a blood sucking bug which can take up to 4 ml of blood in one meal. It is best known for its use in xenodiagnosis (cfr. supra, latent period) of Chagas’ disease.

Following WHO recommendations in patient with latent infection (indeterminate), 2 or 3 different positive serological tests are required before ascertaining the diagnosis of Chagas disease.

**Prognosis**

In an endemic region an asymptomatic person with positive serology is probably a carrier (xenodiagnosis positive in 50 to 100% of cases). The percentage of seropositive persons who develop symptoms is highly dependent on the geographical region (e.g. 10 to 30%). Some people have mega-organisms but are asymptomatic.

Chagas’ disease variables associated with adverse outcome

- 2 points: Male
• 2 points: Low QRS voltage on ECG
• 3 points: Non-sustained ventricular tachycardia on 24-h Holter monitoring (run of 3 or more consecutive VES, with a frequency >100).
• 3 points: Left ventricular systolic dysfunction: segmental or global wall-motion abnormality on echocardiography (quid apical aneurysms, intracavitary thrombus)
• 5 points: NYHA III or IV
• 5 points: Cardiomegaly present on CXR, defined as a cardiothoracic index > 0.5

Results:

• < 6: low risk 14% mortality rate in 10 years
• 7-11: intermediate risk 44% mortality rate in 10 years
• 12-20: high risk 84% mortality rate in 10 years

**Treatment**

**Acute phase**

The acute phase lasts up to 60 days. All patients who are in this phase should be treated.

**Congenital infection**

All infected children should be treated. The earlier therapy is begun, the better the results.

**Chronic phase**

Etiological drug treatment is indicated for “recent” chronic infections (a few years). In practice all children younger than 10 years are treated. If mega-oesophagus is already present the dysphagia should be treated (the passage and absorption of oral medication may be severely impeded). Etiological treatment in these latter patients was not advised formerly but more recent data have brought this into question. In a study in Argentina, 131 patients with chronic Chagas’ disease were treated with benznidazole. After an average follow up of 8 years, 4.2% exhibited ECG changes compared to 30% in the untreated group. There was also considerably less clinical deterioration in the treated group (2.1% compared to 17%).

The results of a large multicenter prospective study (the BENEFIT study) has however recently
demonstrated that an etiologic treatment with benznidazole did not provide any clinical benefit when a patient with chronic infection had already developed a cardiomyopathy (no reversibility). Treating this group of chronic patients appear to be futile while exposing them to some drug toxicity. Whether this is also true for a patient with latent infection and no complication (yet) will require additional evaluation. Such studies are however very difficult to conduct due to the very long latency period to obtain robust clinical outcome data.

**Accidental infection**

This may occur for example in laboratory staff. A serum specimen should be frozen before beginning treatment and a second blood sample taken 4 weeks later. Serology is performed on these paired sera. Benznidazole 7-10 mg/kg/day x 10 days is the usual treatment regimen in this situation.

**Transplant patients**

There are two possible situations: transplantation of an infected organ into a non-infected patient and transplantation of a healthy organ into an infected patient. A donor may be infected so that the recipient becomes infected. Normally the donor is tested beforehand and positive donors are refused, but nevertheless these situations sometimes occur. Alternatively transplantation may be carried out on a patient who is a chronic carrier. The immune suppression that these patients undergo [steroids, azathioprine (Imuran®), tacrolimus (Prograft®) and cyclosporine (Sandimmun®)], may lead to reactivation of Chagas’ disease. In both cases treatment with benznidazole 5 mg/kg/day x 60 days, is indicated.

**HIV patients and Chagas**

Infection with HIV may lead to significant flare-up of Chagas’ disease. In endemic regions all HIV patients should be monitored for Chagas’ disease. If positive, benznidazole is recommended. There is insufficient data concerning chemoprophylaxis. Since the initial step is often serology, one would normally first try to confirm the diagnosis with a second serological test (ELISA-based preferably) and by looking for circulating parasites by microscope (QBC or buffy coat), PCR and perhaps xenodiagnosis. If the diagnosis is confirmed, the patients deserve to be treated as their risk of severe complications (cardiac, digestive or CNS) is high. Benznidazole is preferred to nifurtimox, since nifurtimox is a treatment that is really badly tolerated in adults (notably a lot of nasty allergic reactions). Benznidazole 5mg/kg (max 300mg) daily for 60 days is not an easy treatment to administer neither (beware of skin toxicity!).
Pregnancy

Treatment during pregnancy is not recommended, although congenital Chagas has been well documented. It is clear that more understanding and better outcomes are sorely needed. Infants of infected mothers have to be carefully followed-up to early detect congenital Chagas.

Treatment: Drugs

There are several problems. The drugs have an unsatisfactory cure rate. The chronic lesions may be caused by auto-immune mechanisms and might not be improved by eradicating parasites (as suggested by the recent BENEFIT trial). Parasites play however some role since the disease worsens during immune suppression as in transplantation and in HIV. The drugs should be given long term (minimum 2 months). Results vary from country to country, possibly due to a difference in parasite susceptibility. Side effects occur more often in adults than in children. It is best to avoid steroids or other immunosuppressive drugs, since these may exacerbate the infection.

Nifurtimox (Lampit®) 5 mg/kg/day orally, slowly increased to 15 mg/kg/day (divided over 3 doses) for 2 to 4 months. There are regular problems for the sustainability of its production. Side effects: neurotoxicity (insomnia, tremor, polyneuritis), nausea, leukopaenia, thrombocytopenia or hypersensitivity. May cause haemolysis in G6PD deficiency [glucose-6-phosphate dehydrogenase]. In the acute phase the parasites disappear from the blood in 80 % to almost 100 % of cases. The actual cure rate is 50-60%. In a prospective study conducted in Switzerland among Bolivian immigrants, more than 90% of the patients developed some side-effects, sometimes severe (angioneurotic oedema, Dressler syndrome) and half had to discontinue the drug before the end of the 2-month therapy.

Benznidazole (Radanil®, Ragonil®, Rochagan®) 5-10 mg/kg/day orally for 1 to 2 months. Administration (generally 100 mg tablets) is twice daily. The same side effects as nifurtimox, but less frequent and less pronounced, although skin rash occurs relatively frequently (up to 30-40% of patients, with probably some genetic predisposition) sometimes accompanied by swollen lymph nodes or angioedema. The pharmaceutical company Roche has donated all commercial rights and the technology to manufacture benznidazole to the Brazilian government. In all countries 2-month treatment is recommended, except in Argentina where experts recommend a one-month treatment only.
Other types of drugs for treatment

Posaconazole, an anti-fungal therapy, was found to be inferior (in terms of parasitological failure) to benznidazole in a randomized control trial in Spain, published in 2014.

Fexinidazole has a clear anti-T. cruzi in vitro activity, but no clinical study has taken place so far.

Ravuconazole is a new triazole with in vitro activity against species of Candida, Cryptococcus and Aspergillus, but also in vitro and in vivo (mice) activity against Trypanosoma cruzi. Ravuconazole has a long half-life in humans, which hopefully will facilitate compliance in patients. Clinical trials for its use in Chagas’ disease are ongoing in Bolivia.

In the chronic phase the usefulness of these drugs could not be demonstrated (BENEFIT Trial) at least in patients having already (mostly heart) complications.

Symptomatic therapy is therefore indicated: oesophageal sphincter dilation, extramucosal cardiotomy (Heller’s operation), colon surgery. An experimental treatment is the endoscopic injection of botulin toxin into the distal oesophageal sphincter (e.g. 20 U into each quadrant).

In heart failure diuretics, ACE-inhibitors and antiarrhythmic drugs may be beneficial. Beta-blockers are best avoided in view of the AV-conduction problems and brady-arrhythmias. Aspirin or anticoagulants are indicated for patients with atrial fibrillation, previous embolic phenomena and apical aneurysms. Amiodarone (Cordarone®) is effective in more than 50% of patients who develop ventricular extrasystoles or ventricular tachycardia. A bifascicular or trifascicular conduction block, also a second or third degree AV-block are contra-indications. A high incidence of “torsades de pointes” has been observed during use of disopyramide and other class I antiarrhythmic drugs. Pacemakers, automatic defibrillators and cardiac surgery (including heart transplantation) are reserved in practice for those with financial means and these persons have an inherently low risk of infection. It is obvious that such costly procedures will not be within the financial means of the average Chagas’ patient.

Prevention

The animal reservoir of Trypanosoma cruzi cannot be eradicated. There is no vaccine. Chagas’ disease is typically a disease of poverty. Improvements in housing (brick or plaster walls, corrugated iron roofs, long-acting insecticides on house walls) diminish the insect population. A mosquito net has also proven usefulness here. Serological testing of the blood used for transfusion is very helpful. To
date the various biological methods of eradication of the vectors (insecticide sprays, increasing natural enemies) which have been tested have not been effective because a new ecological balance is very quickly achieved but have brought substantial control in most regions.

In 1991-92 the “Southern Cone Initiative” project was launched by Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay with the objective of stopping the transmission of Chagas’ disease. In 1997 Peru joined the project. After an initial phase for preparation (charting the foci, programming the activities, calculating the costs) there was an attack phase with insecticides, repeated after 3 to 6 months. Insecticide-containing paint is cheaper than the traditional insecticides which are applied by spraying. Insecticides dispersed by fumigant canisters were also used. These are locally produced e.g. in Argentina, are cheap, effective and also active against *Aedes aegypti*, the important dengue vector. At present there are effective colourless long acting insecticides. The fact that people see the bugs, cockroaches, etc. lying dead after spraying is a bonus which makes it easier to accept the spraying procedure. In the Southern Cone Initiative, 1,800,000 houses were treated with pyrethroids (deltametrine, lambda-cyhalothrine, cyflutrine) by the year 2000.

Since then there has been further selective treatment of the houses which still exhibited infestation with triatomes. Simple “sensor boxes” of cardboard (traps for the bugs) were placed in the rooms and the occupants themselves could simply ascertain the presence of triatomes. The last phase is surveillance for the detection of residual foci. This is decentralized and involves the population. The effectiveness of the control program has been demonstrated by the very pronounced drop in seropositivity among young children. The surveillance phase has been reached in 6 countries of the Southern Cone. At present there are several South American countries (Colombia, Ecuador, Venezuela) which have a national control program. Similar programmes were begun in Central America in 1997: Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Mexico and Panama. These programs can only be successful if there is participation of the population and if they can be continued for long enough. The latter is a political decision.

In July 2007 the WHO Global Network for Chagas Disease Elimination was launched in order to coordinate global efforts to eliminate this disease. It includes also many non-endemic countries (such as Spain or USA) where Chagas disease in Latin American immigrants have given rise to a substantial number of secondary transmission (by blood transfusion or transplantation), requiring locally adapted control efforts (screening).
Amoebiasis - General

General

Amoebiasis in our context means infection with *Entamoeba histolytica*. This is a unicellular cosmopolitan parasite. The first description of the parasite was in 1875 by Fedor Lösch in St Petersburg. This concerned an infection in a young Russian farmer in Arkhangelsk, 150 km from the Arctic circle. This illustrates the fact that the infection is not restricted to the tropics. Transmission depends on the level of sanitation and faecal hygiene in a country or region.

*Entamoeba histolytica* trophozoite in rectal mucosa. Copyright ITM
Entamoeba histolytica cysts. Cysts never contain red blood cells. Copyright ITM

Pathogenicity of Entamoeba histolytica

There was considerable confusion concerning the nomenclature and pathogenic properties of *Entamoeba histolytica*. It is now recognized that there are morphologically identical amoebae, some of which are non-pathogenic and some of which are pathogenic. This concept was introduced in 1925 by the French parasitologist Emile Brumpt. The non-pathogenic amoebae are called *Entamoeba dispar*. This should also not be confused with other completely non-pathogenic species, including *Entamoeba hartmanni* (previously sometimes called “small race” *E. histolytica*). In 1978 it was discovered in London that the two kinds of amoebae could be differentiated using isoenzymatic electrophoresis. Pathogenic amoebae always belong to one group and non-pathogenic amoebae always belong to the other group. In 1989 it was discovered that *E. dispar* always differs from *Entamoeba histolytica* by well-determined genetic (DNA) markers. Non-
pathogenic *Entamoeba dispar* never changes into pathogenic *Entamoeba histolytica*. Earlier reports of this appear to be due to laboratory errors: mixed cultures and/or contamination of cultures in the lab. In pathogenic *Entamoeba histolytica* isolates with low virulence and with high virulence can be seen (virulence is a measure of the severity of illness which certain strains can cause in certain circumstances). The degree of virulence is variable, because this is determined by several parameters, including the environment (in contrast to properties which are genetically determined). Isolates with low virulence are non-invasive, while isolates with a high degree of virulence are invasive.

**Motility of Entamoeba histolytica**

*E. histolytica* trophozoites are highly motile. The fuel for this constant motion comes from the anaerobic conversion of glucose and pyruvate to ethanol. *E. histolytica* has no mitochondria (probably through secondary loss). Many of its metabolic enzymes seem to be of prokaryotic origin, possibly acquired from the lateral transfer of genes from bacteria.

**Life Cycle and transmission**

Infection is caused by ingestion of *E. histolytica* cysts. One cyst develops in the small intestine into 8 motile trophozoites (one trophozoite with 4 nuclei divides 3 times and each nucleus divides once to produce 8 trophozoites from each cyst) which then find their way into the colon. The trophozoites multiply by asexual reproduction and in turn produce cysts, which are then excreted with the faeces. The cyst is quite resistant and can survive for a long time in the outside world. Excreted trophozoites die quickly and therefore are not responsible for transmission. Cysts of *E. histolytica* are never found in tissues. The parasite is transmitted feco-orally as a cyst, usually from person to person. Transmission via water also occurs. Dogs, cats, rats, pigs and monkeys may become infected but do not form a significant animal reservoir (Note: kittens were used by E. Brumpt as a very susceptible animal model to test the pathogenicity of amoebae). Flies and cockroaches may carry cysts. Their role in transmission has not been properly investigated but is probably of minor importance. The main source of infection is humans. Amoebiasis is thus not a zoonosis. Infection via sexual intercourse is rare (via anal contact). The latter method of transmission may result in severe and mutilating lesions of the genitals.

*Entamoeba histolytica* is considered to be an asexual organism, but many mysteries persist. Some pieces of evidence don’t fit with this asexual idea, such as the appearance of putative heterozygous populations after mixing homozygotic populations for certain isoenzym classes. Also, *E. histolytica* has
the full complement of meiosis genes, which one would expect to have decayed over time if the organism abandoned the sexual life cycle.

**Prevention**

Amoebic cysts are resistant to normal chlorination of drinking water. Boiling and filtering drinking water eliminates the parasite. Large scale prevention depends mainly on improved sanitation and hygiene. No vaccine is available. Amoebiasis is not an opportunistic infection in HIV patients.
Entamoeba histolytica Life Cycle (courtesy of CDC)
Intestinal amoebiasis

Clinical aspects

We can differentiate 4 different situations in intestinal amoebiasis:

- asymptomatic carriers
- amoebic colitis
- fulminant colitis
- amoeboma

Asymptomatic carriers

Cysts can sometimes remain in the intestinal lumen for years without causing any damage: the patient is then an asymptomatic carrier. The majority (90%) of patients fall into this group. Asymptomatic carriers have by definition no symptoms of amoebiasis. These persons can be detected by faeces analyses. This may show cysts of non-pathogenic *E. dispar* or of potentially pathogenic *E. histolytica*, which for unknown reasons is not invasive. Differentiation with cysts of *Entamoeba coli* (which are larger and have 8 nuclei) is important. *Entamoeba coli* is not pathogenic.

Amoebic colitis

The incubation period of amoebic colitis varies greatly. When *Entamoeba histolytica* penetrates the intestinal mucosa (becomes invasive) it produces ulcerations of the colonic mucosa [Gr. histo-lytica, i.e. referring to breaking down tissues]. The ulcerations are sharply defined and have eroded undermined edges. This is expressed clinically as abdominal pain, diarrhoea with blood in the faeces, and only moderate or no fever, with good general condition. When the rectum is affected there is tenesmus (painful cramps in the anus). Peri-anal ulcers may occur via direct spread from rectal amoebiasis. The ulcers develop rapidly and are painful. After suffering from amoebic colitis there may be persistent intestinal problems, the aetiology of which is unclear.
Entamoeba histolytica rectitis, with spread to the perianal skin. Copyright prof Gigase, ITM
Entamoeba histolytica colitis. Notice the typical skipping lesions. Copyright ITM

**Fulminant colitis**

There is sometimes a fulminant course with high fever, a severely ill patient, intestinal bleeding or perforation of the colon. A slow seepage of intestinal content into the peritoneum is very likely in a severely ill patient whose condition deteriorates progressively, together with the formation of ileus (intestinal paralysis) and a distended abdomen. A fulminant course may occur if patients are treated with steroids or other immunosuppressive drugs (e.g. if amoebic colitis is wrongly thought to be Crohn’s disease or haemorrhagic ulcerative colitis) and sometimes in very young children and elderly.

**Amoeboma**

In 1% of patients an inflammatory thickening of the intestinal wall occurs. A mass may then be palpated (amoeboma). The diagnosis may be made via biopsy. The inflammatory mass may mimic colon carcinoma. Countless trophozoites are found in the tissues (never cysts). Correct therapy produces a pronounced reduction in the volume in approximately 3 days.

**Diagnosis**

When amoebic dysentery is suspected, a fresh faecal sample or a swab from a rectal ulcer should be examined under a microscope. If examined quickly (a fresh stool, still warm) the colourless motile trophozoites can be seen. Motility disappears when cooled, and the parasites are then difficult to recognize. They should be differentiated from actively motile macrophages. The trophozoite (motile form) has one nucleus. When colourless this nucleus is scarcely if at all visible. Once stained the nucleus is moderately visible. Lugol staining kills the parasite almost immediately (motility disappears). Stained *Entamoeba histolytica* trophozoites have a transparent outer border (ectoplasm) and an opaque inner border (endoplasm). The trophozoite measures 20 to 40 µm and may contain red blood cells (unlike other amoebae). The last detail is probably pathognomonic for pathogenic *Entamoeba histolytica*, but is not always present and this statement is contested by some.
Entamoeba histolytica trophozoite. Morphologically, it is only possible to differentiate Entamoeba
dispar from E. histolytica if the trophozoite contains engulfed red blood cells. Only E. histolytica is
haematophagous, although this statement is contested. Copyright ITM
The cysts have 1, 2 or 4 nuclei and measure 8 to 15-20 µm. The nuclei are best revealed by means of an iodine stain. They have a dark circumference and a dark central point (karyosome), these features are helpful in distinguishing with non-pathogenic species such as *Entamoeba coli*. Iodine staining can also detect glycogen (brown) in young cysts. Fresh cysts of *Entamoeba histolytica* also contain what are called chromatoid bodies. These are squat, oval inclusions which can easily be detected (black) with an iron-haematoxylin stain (not with iodine stain). They are not present in *Entamoeba coli* or *Endolimax nana* cysts. In active dysentery, often no cysts are found in the faeces, but if there is little diarrhoea, the parasites have time to encyst. Since excretion of the parasites is intermittent, it is best to carry out 3 different stool analyses before deciding upon a negative result.

Antigen detection is sensitive, specific, rapid, easy to perform and can distinguish between E. histolytica and E. dispar. Stool and serum antigen detection assays that use monoclonal antibodies to bind to epitopes present on pathogenic E. histolytica strains (but not on non-pathogenic E. dispar
strains) are commercially available for diagnosis of E. histolytica infection. Detection of parasitic DNA or RNA in faeces via probes can also be used to diagnose amoebic infection and to differentiate between the different strains. PCR is about 100 times more sensitive than faecal antigen tests.

**Intestinal amoebiasis: Differential diagnosis**

The intestines may contain several species of harmless commensal amoeba. Differentiation with these other non-pathogenic amoebae is important; they include:

*Iodamoeba butschlii*: mononuclear cysts, big glycogen supply

*Entamoeba hartmanni*: small cysts with four nuclei

*Endolimax nana*: smaller round or oval cysts with 2-4 nuclei (measuring 6-12 µm) and slow-moving trophozoites (L.: limax = slug)

*Entamoeba coli*: larger cysts containing 1, 2, 4 or 8 nuclei

*Entamoeba dispar* is a special case (see above)

In dysentery it is important to distinguish between bacillary and amoebic dysentery since their treatment is completely different. A diagnosis may be made clinically but it is best to confirm this by microscopy as there is partial clinical overlap of the two diseases.

*Balantidium coli* is a pathogenic ciliate which can cause severe colitis. This illness is very similar to intestinal amoebiasis and the diagnosis can only be made by faeces examination. Treatment is with tetracyclines or metronidazole.

Pseudomembranous colitis is caused by infection with toxicogenic *Clostridioides difficile*. These bacteria can be selected out and can proliferate after administration of certain antibiotics. Metronidazole is a good treatment in this case. Vancomycin is equally effective but will not be given in third world countries in view of its high cost. A related bacterium, *Clostridium perfringens*, can cause necrotizing colitis (necrotic enteritis, Pigbel syndrome). This disorder has an acute course and is very severe.

Sometimes gonococcal proctitis or lymphogranulomatosis venereum (due to *C. trachomatis*) can be confused with amoebiasis. There are then no proximal intestinal lesions and culture of the mucus or
PCR methods provide a diagnosis. Crohn’s disease and ulcerative colitis are rare in the tropics. Radiology and biopsies are essential for their diagnosis.

<table>
<thead>
<tr>
<th>Bacillary dysentery</th>
<th>Amoebic dysentery</th>
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<tbody>
<tr>
<td>Acute onset</td>
<td>Gradual onset</td>
</tr>
<tr>
<td>Poor general condition</td>
<td>General condition normal</td>
</tr>
<tr>
<td>High fever</td>
<td>Little fever (adult)</td>
</tr>
<tr>
<td>Severe tenesmus</td>
<td>Moderate tenesmus</td>
</tr>
<tr>
<td>Dehydration frequent</td>
<td>Little dehydration (adult)</td>
</tr>
<tr>
<td>Faeces: no trophozoites</td>
<td>Trophozoites present</td>
</tr>
<tr>
<td>Faecal culture positive</td>
<td>Faecal culture negative</td>
</tr>
</tbody>
</table>

**Treatment**

**Asymptomatic carriers**

Since high percentages of the population may be cyst carriers (e.g. 10%) there is little point in treating cyst carriers found by chance in an endemic region. In any case, 90-95% of these people are infected with the non-pathogenic *Entamoeba dispar*. If this is nevertheless desired (e.g. in people who prepare food) paromomycin (Gabbroral®, Humatin®) is indicated. Diloxanide furoate (Furamide®) and iodoquinol (Intetrix®) can be used. In regions of low endemicity it may make sense to treat asymptomatic carriers to prevent transmission and to prevent possible development of later invasive amoebiasis (even if this risk is low). 5-Nitro-imidazoles are not effective against cysts.

**Amoebic colitis**

Parasites in the tissues (intestinal wall) can be treated with nitro-imidazoles, such as metronidazole, secnidazole, ornidazole or tinidazole. Secnidazole has the longest serum half-life (17h) compared with 12-13h for tinidazole, 11h for ornidazole and 8h for metronidazole. The dose of metronidazole (Flagyl®) is 500 mg q.i.d. for 5 or more consecutive days (adults). Tinidazole (Fasigyn®) is more expensive but has fewer side effects. Two grams per day x 3 days is sufficient for amoebic colitis.
Ornidazole 500 mg b.i.d is given for 5 days. Alcohol is forbidden during treatment due to disulfuram effect with severe nausea. These drugs are rapidly absorbed in the proximal intestine. For this reason, they are insufficiently active upon the parasites in the distal intestinal lumen.

The latter are treated with paromomycine (Gabboral®, Humatin®) 10 mg/kg or 500 to 750 mg t.i.d. for 7 days. These drugs are not active against parasites in the tissues. The two drugs thus complement each other. An alternative contact amoebicide is diloxanide furoate (Furamide® = a contact amoebicide). Dose: Furamide® 500 mg t.i.d. for 10 days (adults). Children: 30 mg/kg/day. Nitazoxanide (Alinia® 500 mg tablets and 100mg/5 ml oral suspension) proved very effective as a tissue amoebicide and as a luminal amoebicide. However it is not readily available and is extremely costly.

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Hepatic amoebiasis

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General

If amoebae are transported with the venous blood from the intestinal wall to the liver, an abscess in the liver may be formed: hepatic amoebiasis. If the abscess is adjacent to the fibrous capsule of the liver, adhesions are formed. A subphrenic abscess is less frequent than direct perforation of the diaphragm with empyema or fistula formation to the bronchi. Perforation to the peritoneum is rare. Perforations of the intestine, biliary ducts or navel with secondary phagedenic ulceration of the skin are more frequent than generalized peritonitis. Abscesses of the left hepatic lobe may perforate the pericardium in a life-threatening manner.

[The term “abscess” is not correct here in the strictest sense as this is not a collection of pus cells (white blood cells). It is local cytolysis of liver tissue.]

Clinical aspects
Liver amoebiasis with perforation of the abscess through the abdominal skin. Photo Prof. Gigase. Copyright ITM

Upon physical examination, there is fever and pain in the liver region (pain upon palpation or percussion). The pain increases during deep inspiration or coughing. If the abscess volume is significant, the liver will be enlarged, and the diaphragm will be elevated (percussion, auscultation, chest X-ray). The patient may develop pain in the right shoulder (referred pain). Dullness upon percussion of the base of the right lung may be due to the elevation of the diaphragm, reactive pleural fluid or breakthrough to the pleura, or atelectasis of the lung. Jaundice occurs in a minority (6-29%) of patients and tends to be a very late symptom. Jaundice can result from biliovascular fistula (with backflow of the bile into the hepatic veins) or compression of bile ducts. The abscess spreads until it breaks through to the surroundings: the pleura (empyema), the lung, the pericardium or the
skin. If fistulisation to the skin occurs, there may be a swift progression of a painful skin ulcer. Untreated amoebic liver abscess is often fatal.

**Diagnosis**

The diagnosis of a hepatic abscess may be suspected from clinical findings. Leukocytosis will be high (and there is no eosinophilia). Ultrasound and serology (ELISA, Latex agglutination) can confirm the diagnosis but are often unavailable. Antibodies will remain present for a long time -often years- after infection. An amoebic abscess of the liver will contain necrotic liver tissue at its center. Upon aspiration, this often has a dark brownish-red color called “anchovy” or “chocolate” pus, but the pus may also be yellow, grey or greenish. The pus has no offensive odor, unlike most bacterial (anaerobic) abscesses, which is an important difference. The abscess wall contains trophozoites, but the necrotic liver tissue does not. Local edema or bulging of the skin with or without fluctuation indicates the proximity of the abscess and the site where a puncture can be carried out. In case of doubt, a trial therapy quickly produces a spectacular improvement. Fewer than 20 % of people with a hepatic abscess have Entamoeba histolytica in the feces. Therefore, the absence of amoebae in the stools does not rule out the diagnosis.
Entamoeba histolytica. Ultrasound of the liver showing an amoebic liver abscess. Copyright ITM
Liver abscess due to infection with Entamoeba histolytica. CT scan of the liver shows a circular necrotic area. Copyright ITM

Hepatic amoebiasis: Differential diagnosis

1. Pyogenic/anaerobic hepatic abscess: stinking pus, poor general condition, often icterus, negative serology, sometimes portal-of-entry in the intestine (e.g. colon tumor, appendicitis).
2. Hydatid cyst: slow development, no fever, no toxemia, serology positive for Echinococcus, sometimes calcifications on abdominal X-ray, no leukocytosis. Ultrasound may show daughter cysts.
3. Biliary cysts: ultrasound shows a thin wall with anechoic content, otherwise asymptomatic.
4. Haemangioma: hyperreflective on ultrasound, otherwise asymptomatic. On CT scans with dynamic sequences, there is a centripetal staining with a delayed isodense appearance to the surrounding liver tissue. On MRI, a haemangioma is extremely hyperreflective on T2-weighted images (T2 = “water images”).
5. Metastases: ultrasound shows generally (but not necessarily) irregular and hyperreflective structure; central necrosis may occur. Frequently peripheral edema.

6. Hepatoma: no fever or toxemia, no response to trial therapy, elevated alpha-feto protein, negative serology, often related to HBV or HCV; biopsy is diagnostic.

**Treatment**

An amoebic liver abscess is treated with metronidazole for 10 days (often initially IV) or tinidazole 2 gr daily for 5 days, followed by paromomycin or diloxanide furoate for 10 days. The latter is to destroy any amoebae in the lumen of the intestines. If the diagnosis is known, aspiration is only carried out for very large abscesses or if there is a risk of breakthrough. Surgery is indicated if the abscess ruptures (e.g. into the peritoneum). If a relapse of the abscess occurs, this usually happens within two months.

**Amoebiasis of other organs**

Amoebiasis of the lungs is generally the result of the spread of an amoebic abscess of the liver, which perforates through to the base of the lung. Breakthrough to a bronchus may occur. The prognosis is usually favorable. Amoebic pleuritis (empyema) is an unpleasant complication because of the need to drain the empyema. Other locations are rare and include:

- Primary amoebiasis of the lung without prior hepatic amoebic abscess.
- Abscesses in muscles, e.g. the thigh.
- Ulceration of the skin of the lower limbs by amoebae could result from superinfections of skin wounds due to scratching with dirty nails.
- Urogenital forms, either due to fistula formation of intestinal lesions to the bladder or of peri-anal ulcers to the vagina and cervix of the uterus. Location on the penis if the partner has ulcers of the vagina/cervix or anal ulcers.
- Parasites may appear elsewhere and lead to abscesses in other organs, e.g. the brain.
Giardiasis

Summary

- *Giardia lamblia* is an unicellular flagellate
- Faeco-oral transmission via cysts
- Sometimes asymptomatic infection
- Sometimes diarrhoea, atypical abdominal discomfort, bloated abdomen
- First-line treatment with nitroimidazoles, by preference tinidazole

General

Giardia lamblia trophozoite in faeces. Copyright ITM
Giardia lamblia (G. intestinalis, G. duodenalis) is a unicellular parasite (flagellate) which causes intestinal infections. The infections are often asymptomatic and Giardia was for a long time thought to be non-pathogenic. Since 1981 it has been regarded as potentially pathogenic and as the cause of diarrhoea and various forms of abdominal discomfort. In developing countries the infection occurs often in children but its frequency diminishes as they grow older. G. lamblia may infect various animals, including dogs, cats and beavers.

The taxonomy of this intriguing species is still to be clarified. At present, seven distinct genetic groups based on protein and DNA polymorphisms can be distinguished in Giardia. Each group has its own host range, with group A and B able to infect humans.

**Biological information**

Giardia has no de-novo synthesis of lipids, which means that the parasite is dependent on
exogenous lipids and bile salts (hence its location in the duodenum).

*Giardia lamblia* is possibly a complex of different species. Chemotaxonomy via determination of antigens using monoclonal antibodies shows that there is significant antigenic variation. DNA-analysis is promising, but *Giardia* has a complex genome. Using iso-enzymatic analysis, 13 zymodemes are known at present.

*Giardia* contains two functionally equivalent and apparently identical nuclei. The two nuclei remain physically distinct during mitosis in the trophozoit. Both nuclei are diploid and transcriptionally active. The two daughters of a single nucleus segregate to different trophozoites. It is still not clear yet at present if *Giardia* is asexual (as traditionally assumed), parasexual (diplomixis: nuclear fusion of the 2 nuclei during encystations, accompanied by homologous recombination without meiosis) or sexual. Till present, *Giardia* has not been caught “in the act” however. If diplomixis occurs this would be unique to *Giardia*.

**Life cycle of Giardia**

Cysts are swallowed with water or food. In the duodenum excystation occurs which releases the trophozoite. This measures 12-18 µm. It attaches itself to the duodenal and jejunal intestinal villi by means of a kind of ventral sucking disk. The parasite reproduces only by asexual division. The trophozoites may multiply until the whole surface of the intestine is coated with parasites. Possibly this mechanical screening off the intestine contributes to malabsorption.

As trophozoites are carried to the more distal parts of the intestine, the parasite encapsulates. The cyst is resistant in the outside world but trophozoites perish. Cysts remain viable in a wet, cool outside environment. They are not very resistant to drying out. The cysts measure 10 x 7 µm. Transmission is via direct faeco-oral contact, food or via water. There is an animal reservoir and this is sometimes involved in human infection (giardiasis is known in Canada as “beaver fever”). In industrial countries dogs and cats are frequently found to be infected but almost always without symptoms.

**Pathogenicity**

In many cases infection is asymptomatic, but some patients develop symptoms. One hypothesis as to the pathogenicity is the mechanical covering of the intestinal epithelium (see above). This is not the only way in which the parasite gives rise to symptoms. *Giardia* is cytopathogenic on cell monolayers in vitro. Probably there is also in-vivo enterocytic damage with secondary disaccharidase (lactase) deficiency. Indeed, villous atrophy is found in patients. Another way in which *Giardia* may be
pathogenic, is the destruction of conjugated bile salts with secondary steatorrhoea. Yet another unanswered question is whether the immune response contributes to the pathogenesis. In vivo *Giardia* has frequent endosymbiotic bacteria up to 100 per trophozoite or so it was thought. This may possibly influence pathogenicity. The same question arises as regards any ectosymbionts. *Giardia* itself can be infected with an RNA virus of unknown clinical significance.

**Clinical aspects**

The disease is asymptomatic in approximately 80% of cases. The clinical spectrum ranges from silent carrier status to a malabsorption syndrome. The incubation time is 1 to 2 weeks. If symptomatic, an undifferentiated acute to subacute diarrhoea which lasts on average 1 to 6 weeks occurs. In some the diarrhoea is steatorrhoeic with malabsorption. This may be accompanied by mild fever, abdominal pain, ructus (“purple burps”), meteorism and anorexia, malaise and vomiting. The diarrhoea may be intermittent, chronic and recurrent, chiefly in patients with an IgA deficiency, hypogammaglobulinemia or agammaglobulinemia. This reflects the fact that secretory immunity in the intestinal lumen is more important for clearance than cell-mediated immunity within the intestinal lumen.

**Diagnosis**

Diagnosis is quite difficult due to the intermittent character of the presence of *Giardia* in the faeces. The diagnosis is mainly based on fresh or enriched faecal preparations. Sometimes several analyses of faecal specimens are needed. One specimen gives a detection rate of approximately 70% while 3 specimens increase this rate to approximately 85%. Generally cysts are found rarely trophozoites. Other techniques such as duodenal aspiration or the EnteroTest (the string test) are less practical. In rare cases infections have been recognised on jejunal biopsy material or mucus sampled during endoscopy. Recent techniques for detecting antigen in faeces have proved sensitive, specific and fast. PCR methods are increasingly available in high-resource settings. Microscopically a differentiation needs to be made with other flagellates such as the commensal *Chilomastix mesnili*, *Enteromonas hominis*, *Trichomonas hominis* (= *Pentatrichomonas hominis*) and *Retortamonas intestinalis*.

The histological intestinal lesions are not very pronounced: flattening of the intestinal villi, lymphocytic infiltration of the mucosa, no ulceration. Radiology of the small intestine is non-specific. If giardiasis is suspected, but cannot be proven a trial of therapy can sometimes be used.
Treatment

*Giardia* is an anaerobic protozoon, which possibly explains its sensitivity to nitro-imidazoles (e.g. metronidazole). The drug of first choice are nitro-imidazoles, especially tinidazole (Fasigyn®), of which 2 grams is to be taken in one dose (adult patient). This gives a cure rate of 90 to 95%. Metronidazole may also be used but produces more side effects. Ornidazole (Tiberal®) 500 mg b.i.d. is an alternative but is best given for 5 days. Alcohol should be avoided since there may be an antabuse effect. Other nitro-imidazoles are also sometimes used: secnidazole (Flagentyl®), nimorazole (Naxogyn®).

Refractory giardiasis possibly related to lower susceptibility/resistance to nitro-imidazoles is increasing. Mepacrine (quinacrine, atebrine) is an old drug (3 x 100 mg/day orally for 5 days) which gives good results if tinidazole fails. It also kills cysts, as opposed to metronidazole. It is a yellow product and may cause a jaundice-like skin discoloration, which the patient should be warned about beforehand. It may also cause haemolysis if there is severe G6PD deficiency. Albendazole has also proved effective in vitro but produces varying results in vivo. Nevertheless it is a good second choice. Paromomycin (Humatin®, Gabbroral®) is an aminoglycoside which has very low absorption when taken orally and is thus active in the intestinal lumen. However there is quite a high relapse rate (25%). Nitazoxanide is an expensive alternative (500 mg BD x 3 days for an adult).

Metronidazole is often available in tropical countries when tinidazole is unavailable. Selective toxicity is achieved because the drug is only reduced in an anaerobic environment (reduction is prevented by oxygen). Its action is limited to anaerobic protista (*Giardia, Entamoeba histolytica, Trichomonas vaginalis*: all three lack mitochondria) and anaerobic bacteria. Side effects of metronidazole include a metallic taste in the mouth, gastrointestinal disturbances: vomiting, nausea, cramps, headache, and a disulfiram (“antabuse”)-effect. Rarer are CNS toxicity, dizziness, drowsiness, lassitude, paraesthesia, pruritus and urticaria. In therapy-resistant giardiasis the questions should be considered as to whether (1) compliance is failing, (2) is there a possibility of counterfeit medication, a growing problem in many countries, (3) if there may be re-infection (e.g. via an asymptomatic cyst carrier), or (4) immunodeficiency, including IgA-deficiency, (5) possibly the presence of a duodenal diverticulum (mechanical reason for relapse, as the concentration of the therapeutic drug might be rather low) or (6) whether this is a genuine problem of resistance. There is in-vitro cross-resistance between the different nitro-imidazoles. If symptoms persist, (7) long-term lactase deficiency or (8) bacterial overgrowth in the small intestine with possible inactivation of nitro-imidazoles by Gram-negative bacteria should be considered.
Prevention

Prophylaxis is difficult both individually and in the community. Giardiasis is much more common than amoebiasis. The importance of giardiasis is underestimated according to some and it is thought to be one of the ten most important parasitic diseases in humans and may be responsible in poor countries of impairment in child growth. Nevertheless there is little connection between the prevalence and the pathology attributed to the infection. In general the treatment of asymptomatic infections in endemic regions is considered unnecessary.

Treatment of large amounts of drinking water (flocculation, sedimentation, filtration and chlorination) is important. Chlorine compounds work best in water with a low pH and a high temperature when the water contains little organic debris.

Alternatives for chlorine and hypochlorite compounds includes chlorine dioxide, ozonation and ultraviolet irradiation. Boiling of large amounts of drinking water is too costly.

Infection with Various Protista

Non-\textit{E. histolytica} intestinal amoebae
Entamoeba coli cyst in faeces. Cysts can obtain up to 8 nuclei. Copyright ITM
Iodamoeba butschlii in faeces. The glycogen mass will stain brown with an iodine stain. Copyright ITM

At least 10 different amoeba species are found in the intestinal lumen or mouth. Some consider all amoebae apart from *E. histolytica* as non-pathogenic commensals, but more investigation is needed to clarify some issues especially regarding *Blastocystis hominis* and *Dientamoeba fragilis*. Pathogenicity is probably due to strain differences that are increasingly investigated. Genetic analysis indicates that *D. fragilis* is actually more closely related to *Trichomonas* than to amoebae.

1. *Entamoeba histolytica*
2. *Entamoeba dispar*
3. *Entamoeba moshkovskii*
4. *Entamoeba hartmanni*
5. *Entamoeba coli*
6. *Entamoeba polecki*
7. *Entamoeba chattoni*
8. **Entamoeba gingivalis**
9. **Endolimax nana**
10. **Iodamoeba butschlii**
11. **Blastocystis hominis**
12. **Dientamoeba fragilis**

**E. dispar** and **E. moshkovskii** are morphological identical with *E. histolytica*. In order to distinguish between *E. histolytica* and *E. dispar* molecular tools such as PCR technology are used. Most antigen-detection tests cannot distinguish the two organisms, although one test (Wampole *E. histolytica* test) uses reagents that differentiate between *E. histolytica* and *E. dispar*. If trophozoites in stool contain RBCs, they are pathogenic *E. histolytica*, but if the trophozoites do not contain RBCs no species identification can be reached. Limited research has been carried out on *E. moshkovskii*. At present there are no good practical tests to distinguish this organisms from the two other look-alikes. Its presence is suspected especially in people who have *E. histolytica*/*E. dispar*-like cysts in the stools, but who test negative for *E. histolytica*/*E. dispar* antigen. *E. moshkovskii* is highly resistant to the current amoebicidal drugs. The existence of these non-pathogenic look-alikes often results in clinical doubt and leads to overtreatment. Infections with non-pathogenic amoebae are much more frequent than infections with pathogenic *E. histolytica*.

**E. hartmanni** is a non-pathogenic intraluminal parasite which can only be distinguished from *E. histolytica* forms by its smaller dimensions.

**Entamoeba coli** is a non-pathogenic organism that is commonly mistaken for a pathogenic *E. histolytica*. Trophozoites move slowly and never contain red blood cells. *E. coli* cysts are larger (10-30 µm) and may contain up to eight nuclei.

**Endolimax nana** is non-pathogenic. The trophozoites are small (up to 10 µm), move slowly with blunt hyaline pseudopods.

**Iodamoeba butschlii** also has small cysts, about 9 µm. These have only one nucleus and a glycogen mass which stains with iodine (Lugol), from which it gets its name.

**Dientamoeba fragilis** is an amoeboflagellate. The fact that it can develop flagella puts it in a different taxonomic group from the above-mentioned amoebae. It is more closely related to *Trichomonas* sp than to *Entamoeba histolytica*. It is a non-invasive intestinal parasite. Many infections are asymptomatic, but it has been associated with non-specific diarrhoea. It is very difficult to
demonstrate with the microscope because the vegetative form is easily damaged (fragilis = breakable). No cyst stage is known and it is unclear if transmission via trophozoites can take place. One hypothesis as to how transmission of such a fragile microorganism is possible is that *Enterobius vermicularis* (pinworms) could function as vectors but solid evidence is lacking. If the faeces cannot be brought quickly to the laboratory (ideally < 10 min), they should be fixed in PVA (polyvinyl alcohol) or SAF (sodium acetate formalin), otherwise the parasite will most likely not be detected. There seems to be wide genetic variability between isolates, e.g. as demonstrated by differences in DNA melting temperature or variability of certain DNA markers. As more information will become available in the future; it is possible we will encounter a scenario like the one with *Entamoeba histolytica* (being pathogenic) and *Entamoeba dispar* (non-pathogenic): i.e. a heterogeneous species with genetic variants that have similar morphologies but different pathogenicities. It is clear that more study is needed. *Dientamoeba fragilis* infections can be treated with a 5-day course of metronidazole, but a single 2-gram dose (adult patient) of ornidazole it is easier and gives less side-effects. Paromomycin and iodoquinol can also be used and actually give higher cure rates.

For *Blastocystis hominis*, see below (separate chapter).

**Balantidium coli**
Balantidium coli is a large protozoon. The trophozoite measures 30-200 µm x 40-60 µm. The whole surface of the trophozoite is dotted with countless cilia. These are very characteristic and because of this, it is classified as a ciliate (compare with Paramecium). Balantidium coli is the only ciliate pathogenic to humans. Transmission occurs from pigs to humans and from human to human in poor hygiene situations, also via water or food contaminated with cysts including poorly cooked pork sausages. As with amoebiasis the infection may be intraluminal and latent or invasive in the intestinal wall and symptomatic. In the invasive forms ulcerations of the intestinal wall are found which are quite similar to those of amoebiasis, with the same complications and the same clinical forms. Liver abscesses caused by B. coli have been observed but are extremely rare. Diagnosis is parasitological by direct stool microscopy or enrichment techniques. In a fresh preparation, B. coli can be very quickly recognised due to its swift manner of propulsion. Under the microscope, the creature is difficult to keep in the field of vision due to its relatively high speed. Treatment is not always simple. Tetracyclines (10 days) have been used as well as imidazoles in high doses.
Flagellum

One important organelle to move in an aqueous environment is the flagellum. A certain group of micro-organisms (flagellates) take their name from the fact that they possess flagella. The term flagellum (L. flagellum = whip) is used, however for two totally different organelles. Some micro-organisms are dotted with myriads of these organelles which work in a coordinated way and which are then called “cilia”.

Cryptosporidiosis

*C. parvum* is the most common parasite in this group in human infections, but *C. meleagrisidis, C. canis, C. muris* and *C. felis* are also found in immune-compromised persons with acute diarrhoea.

Biological classification

Cryptosporidia are coccidia and belong to the Apicomplexa phylum. Coccidia form an order of unicellular eukaryotic micro-organisms, which includes the following human pathogens: *Toxoplasma gondii, Sarcocystis* sp., *Cryptosporidium parvum, Cyclospora cayetensis, Isospora belli*. Microsporidia do not belong to the Coccidia and form a totally different taxonomic group. DNA analysis of *Cryptosporidium* suggests that there could be more than twenty different species.
Transmission to humans occurs from calves, dogs and cats. Transmission via drinking water or via insufficiently chlorinated water in swimming pools happens frequently. This species is resistant to standard chlorination. The parasite was first observed in humans in cases of persistent diarrhoea in patients with immunosuppression and since 1981 in cases of AIDS. Since 1983 the infection has frequently been recognized as a cause of benign and brief diarrhoea, both in children and adults, and it is one common aetiologies of travellers’ diarrhoea.

**Life cycle details**

The complete cycle of the parasite, sporogony and schizogony, takes place in the same host. People become infected by swallowing thick-walled, resistant oocysts. Once in the intestine the parasites excyst and release sporozoites. They penetrate epithelial cells via the apical membrane.
After maturation of the sporozoite there is asexual reproduction via schizogony with the formation of merozoites. These may either penetrate a new epithelial cell to repeat the cycle (type 1 merozoites) or undergo further intracellular changes (type 2 merozoites) to the sexual form of the parasite. The macrogamont is the female form, the microgamont the male form. The microgamont releases microgametes. After fertilisation and the formation of zygotes, thin-walled oocysts are produced, which after meiosis release sporozoites in their turn which amplifies the infection (auto-infection). Thick-walled oocysts are released into the lumen of the intestine, and are directly infectious via the faeco-oral route. C. parvum induces apoptosis in epithelial cells.

The parasites may be found throughout the entire digestive tract and even in the mucosa of the respiratory tract but are usually limited to the duodenum and jejunum. The incubation period is 4 to 12 days (usually 7-10 days) and is followed by moderately severe diarrhoea without fever usually and with little abdominal pain. Asymptomatic infections may occur. If there is no underlying immunosuppression, spontaneous recovery occurs within a few weeks. It is estimated that 4 to 10% of all cases of diarrhoea in children in tropical environments can be attributed to Cryptosporidium. In patients who have a deficiency in cellular immunity (such as in HIV infection), the diarrhoea is more pronounced can be chronic for several months and recurrent. Fulminant infection with cholera-like diarrhoea may occur in patients with fewer than 50 CD4 T-cells/mm\(^3\). Sometimes the protista enter the biliary tract, resulting in sclerosing cholangitis, strictures and papillary stenosis. Diagnosis is difficult and requires invasive procedures such as retrograde cholangiography (ERCP [endoscopic retrograde cholangiography]). A biliary tract reservoir may contribute to the chronic course of infection. Diagnosis is based on looking for the parasite in the faeces on smears stained with modified Ziehl-Neelsen or Kinyoun staining. The small dimensions of the parasites and their similarity to yeast cells were responsible for the fact that infection in humans was only recognised in 1976. The parasites can easily be recognised on intestinal biopsy material obtained by endoscopy. There are other diagnostic techniques, such as immunofluorescence, antigen-capture ELISA and PCR, but these are not available in most tropical settings. Treatment is mainly symptomatic and can be quite difficult in AIDS patients. The best practical method in these patients is via HAART (highly active antiretroviral therapy). Paromomycin (Humatin®, Gabboral®) is a non-absorbed aminoglycoside and is of limited use. At present the drug of choice is nitazoxanide (Alinia®, Cryptaz®, 500 mg tablets or syrup) but this drug is unaffordable. Cryptosporidium cysts are very resistant to chlorination (much more so than Giardia cysts, although even those have a certain resistance to standard concentrations in drinking water).

**Cystoisosporosis**
Isospora belli mature oocyst containing two sporocysts. Copyright ITM

_Cystoisospora belli_ is a coccidian parasite of the duodenum and proximal small intestine (jejunum) in humans. It is cosmopolitan but more frequent in a tropical environment. The previous name was _Isospora belli_. No reservoir hosts other than man are known. The oocysts are very resistant to environmental conditions and may remain viable for months if kept cool and moist. The sexual and asexual cycles occur in the same host. The parasites are located intracytoplasmic, unlike _Cryptosporidium_. There is a prepatent period of about 9-10 days. Infection may be latent or lead to diarrhoea for one to two weeks occasionally with mild fever, headache, malaise and abdominal pain. The stools tend to be soft, watery or foamy, with an offensive smell, suggesting malabsorption. In immunosuppressed people the infection can become chronic. In such cases oocyst shedding can continue for years. Diagnosis is difficult and is based on stool examination, and biopsy of the duodeno-jejunal mucosa, in which the parasites are not very numerous. Charcot-Leydig crystals (derived from eosinophils) are occasionally found in stools samples of isosporiasis cases.
The infection can be severe and prolonged in case of immunosuppression (in particular in AIDS). It is like cryptosporidiosis a frequent cause of prolonged/recurrent cholangitis in AIDS patients through invasion of the biliary tract.

The condition can be treated with cotrimoxazole (e.g. Bactrim forte® 4 x 1 tablets/day for 10 days). If there is diminished sensitivity or resistance, either pyrimethamine (Daraprim®) 25 mg/day x 20 weeks or the combination ornidazole (e.g. 2 gram on day 1, 15, 30) with albendazole (400 mg BD x 30 days) is used. Ciprofloxacin is also moderately effective (70% cure rate).

**Cyclospora cayetanensis**

*Cyclospora cayetanensis* oocyst unstained

Cyclospora cayetanensis in faeces, unstained. The parasite is about double the size of
Cryptosporidium parvum. Copyright ITM

*Cyclospora cayetanensis* is a protozoon which belongs to the Coccidia. The name is derived from the morphology (the sporocysts are spherical) and from a Peruvian university (most of the epidemiological and taxonomic work has been carried out at the Universidad Peruana Cayetano Heredia, Lima, Peru). Distribution is probably cosmopolitan, but the species is only common in regions with poor hygiene. Protista can be detected in surface water with special techniques. No reservoir is known to date.

After swallowing mature (i.e. sporulated) oocysts, there is excystation after contact with bile salts. The released sporozoites penetrate the jejunal enterocytes. Infected persons eliminate non-sporulated oocysts in their faeces. Until they sporulate, which takes days or weeks these parasites cannot infect a new host. This delay makes direct human to human transmission unlikely.

The protista are present in the duodenum and jejunum and cause persistent watery diarrhoea, often accompanied by significant abdominal discomfort, nausea, tiredness and anorexia and sometimes with mild fever. The symptoms may last several weeks. In particular, non-immune persons such as travellers or small children, will be symptomatic. Cotrimoxazole is used in treatment. This protozoon also causes persistent diarrhoea in HIV-positive persons. If patients cannot tolerate cotrimoxazole, the rather less effective ciprofloxacin may be used.

**Sarcocystosis**

**General**

*Sarcocystis* species are parasites of mammals, birds and reptiles. Human sarcocystosis (syn. sarcosporidiosis) is rarely diagnosed. For some species humans are the definitive host i.e. the host in which sexual reproduction (gametogony followed by sporogony) is completed. In this case there is intestinal sarcocystosis. Humans may also act as accidental dead-end intermediate hosts – where asexual reproduction (schizogony) takes place – for several other species and in these cases there is muscular sarcocystosis.

**Intestinal sarcocystosis**
Intestinal Sarcocystis sp.
Sarcocystis, pseudocyst in muscle. Copyright ITM

*Sarcocystis bovihominis* and *Sarcocystis suihominis* are parasites of humans. Infection occurs due to eating raw or insufficiently cooked meat from cattle or pigs containing tissue cysts (intestinal infection cannot be triggered by the ingestion of sporocysts). The sexual cycle takes place within the cytoplasm in the cells of the human intestinal mucosa. The sporocysts which are released with the faeces are infectious for the intermediate host. These infections are cosmopolitan and generally asymptomatic. They can nevertheless trigger enteritis with peripheral hypereosinophilia. The diagnosis is based on faecal examination. Sometimes the parasites will be detected in surgical resected intestinal specimens. Gastro-intestinal disease is often self-limiting and does not need treatment. There is no known effective treatment.
Muscular sarcocystosis

Muscle infection is caused after swallowing sporocysts (faeces of an infected predator). Each sporocyst releases 4 sporozoites. These penetrate the intestinal wall. Reproduction begins in the vascular endothelium. After dissemination of merozoites there is invasion of skeletal and cardiac muscle tissue and possibly the central nervous system (in animals). The merozoites develop first to metrozoites and then to cystozoites. These tissue cysts remain dormant until the host is eaten by a predator after which the intestinal cycle begins. The tissue cysts gave the genus its name (Gr. sarx = flesh).

Most human infections are apparently asymptomatic. It is also possible that the diagnosis is systematically missed (data from investigation of routine autopsies). No cases of neurological involvement in human patients are known. Some patients with muscular sarcocystosis develop an eosinophilic myositis. The myositis is characterised by muscle pain, painful mild muscular swelling, mild fever, general weakness, bronchospasms and eosinophilia. This should be differentiated from trichinosis (*Trichinella spiralis*). Eosinophilic fasciitis, toxoplasmosis, polymyositis, dermatomyositis and polymyalgia rheumatica may lead to similar clinical pictures. Diagnosis is made via muscle biopsy. The intact cysts in the muscle generally do not trigger a local inflammatory reaction. Dead and ruptured cysts may cause inflammation. Muscular sarcocystosis can be treated with cotrimoxazole, although its efficacy is not proven. The use of corticosteroids is under discussion but often necessary to control the symptoms of myositis when they are prominent.

Microsporidiosis

General
Microsporidia in muscle of AIDS-patient. Ziehl-stain. Copyright ITM
Species belonging to the phylum Microspora are called microsporidia. At present more than 140 genera are recognized and 1200 species have been described. These obligate intracellular organisms appear to have separated very early from the eukaryotic family tree. They have true nuclei, but no mitochondria or peroxisomes. Their ribosomes are prokaryote-like (70S). Since the spore wall contains chitin some researchers regard them as aberrant fungi. They are obligate intracellular parasites and are recovered in countless widely varying host groups (insects, fish, rodents, and so on). Species which can parasitise humans are very small (1-2 µm).

**DNA structure, ultrastructure and life cycle**

*Encephalitozoon cuniculi* holds the record at present for the smallest eukaryotic genome (<2.9
Other species known to infect humans are Brachiola vesicularum, Encephalitozoon cuniculi, Encephalitozoon hellem, Encephalitozoon intestinalis (previously Septata intestinalis), Enterocytozoon bieneusi, Microsporidium africanum, Microsporidium ceylonensis, Nosema algerae, Nosema connori, Nosema ocularum, Pleistophora sp, Trachipleistophora hominis, Trachipleistophora anthropophthera and Vittaforma corneae (previously Nosema corneum). These organisms have mainly been described in immunodeficient persons.

The parasites have a very characteristic ultrastructure. The organism forms oval-shaped spores with an external exospore (glycoproteins) and an internal endospore (chitin). Within the spore is a coiled spiral tube (polar tube). After it is ingested, the spore is stimulated to protrude this polar tube which then penetrates a host cell. The sporoplasm is then injected via this tube into the cytoplasm of the host cell. Subsequently there is reproduction of the parasite (merogony and sporogony). New spores may infect other neighbouring cells or be passed to the outside world to infect a new host.

**Transmission**

Transmission is chiefly via the faeco-oral route but much is still uncertain. Possibly transmission is via aerosol for those protista which cause corneal lesions. Transmission via infected water is being investigated.

**Clinical aspects**

Symptoms will be determined by the anatomical location of the parasites. Disseminated infections, corneal infections (keratitis), intestinal locations etc all occur, almost exclusively in immunosuppressed individuals. In HIV patients with low resistance (CD4 < 100/µL) there is often persistent diarrhoea, abdominal pain, loss of weight and sometimes sclerosing cholangiopathy.

**Diagnosis**

Diagnosis by light microscope (faeces, biopsies, corneal scraping) is often difficult due to the small dimensions of the parasites and the labour-intensive staining techniques. Experience is essential and the parasites must be properly differentiated from fungal spores and bacteria. Electron microscopy is a good technique for species identification, together with PCR but of course this can only be carried out in specialized centres. The organisms can be detected in routine formail-fixed and paraffin-
embedded tissues.

**Treatment**

There is still too little known about treatment. Fumagillin is a product originating from *Aspergillus fumigatus* which is used in microsporidiosis of honey bees, has been used topically in keratitis with good results. Other drugs have been used with varying success. Albendazole is effective in infections with *Encephalitozoon intestinalis* and to a lesser extent in *Enterocytozoon bieneusi*. Nitazoxamide possibly has a place in treatment. Improving immunity in HIV patients, e.g. by combination antiretroviral therapy, often leads to remission of the infection. For symptomatic treatment (e.g. in persistent diarrhoea without knowing its cause), loperamide (Imodium®), opioids (laudanum) or even somatostatin analogues may be used. The latter is of course not easily available in developing countries.

**Blastocystosis**

*Blastocystis hominis* is a rather common enteric unicellular protista. The parasite colonises chiefly the caecum and to a lesser extent the distal colon.
Very little is known of the basic biology of this organism, including the life cycle. Several morphological forms have been recognized: ameboid, vacuolar, avacuolar, multivacuolar, granular, cyst. Which of the forms is responsible for transmission is not known. The vacuolar stage divides, while the ameoboid stage might be invasive and is capable of budding. B. hominis forms pseudopods, and ingests bacteria and debris. It reproduces by binary fission or sporulation.

Transmission is faeco-oral through contaminated food or water. There seems to be a large animal reservoir. Its pathogenicity is controversial. Several studies using different methods and examining different patient groups have reported very variable results, from asymptomatic infection, acute symptomatic infection and chronic symptomatic infection; with abdominal pain, diarrhoea,
constipation, irritable bowel, fatigue, skin rash, and other symptoms. The variation in results led to disagreements concerning a possible pathogenic role of Blastocystis in humans. Maybe Blastocystis has several variants which differ in their pathogenicity or virulence. The pathogenicity might depend on the parasitic load (more than 5 Blastocystis per 40x field, but different pathogenic properties of different strains will likely also play a role). Molecular typing has revealed extensive genetic diversity in morphological identical strains. According to current PCR-based genotype analysis there may be 12 different species which are lumped together under one name. It is possible that additional studies will show that what we call Blastocystis hominis will turn out to be a mixture of different microorganisms, a situation similar to the past confusion about the morphological identical Entamoeba histolytica, E. moshkovskii and E. dispar.

Classically Blastocystis is considered to be non-pathogenic and doesn’t need treatment. However a treatment can be justified if symptoms are severe in the absence of other pathogens or in immunosuppressed patients (AIDS), or if the parasitic load is very high. If considered necessary, metronidazole/tinidazole or trimethoprim/sulfamethoxazole are used for treatment.

**Rhinosporidiosis**

Rhinosporidiosis is an infectious disease which occurs in the New World, Europe, Africa and Asia, but is most common in the tropics (India and Sri Lanka). The disease is characterised by slow-growing, painless polyps or tumour-like masses, which are usually found on the nasal mucosa, lachrymal sac, conjunctivae, palate, larynx or penis. Chronic rhinitis and/or epistaxis may occur. Treatment consists of surgical excision, but recurrence can be expected in approximately 10% of patients. No natural reservoir is known. It is also assumed that people become infected by swimming in fresh water lakes or rivers. It is likely that fish or other water creatures are the normal hosts.

**Protothecosis**

Protothecosis is a rare infection in humans. Infection is more common in cases of immunosuppression (AIDS, leukaemia). The disease is caused by Prototheca wickerhamii and P. zopfii (segbwema). These are aerobic unicellular round (P. wickerhamii) to oval (P. zophii) algae which activity belong to the Chlorococcales [Chlorophyta or green algae]. However they contain no chlorophyl and are colourless. The protista occur in still water, sewage sludge, mud and slime on trees.
Various animals may be infected (cattle, dogs, rabbits, mice, rats, pigs, deer). Humans are infected via traumatic inoculation of the germ into the skin or via infection of an open wound. Infection is usually limited to the skin, where local painless granulomatous hyperkeratotic dermatitis results. Bursitis and tenosynovitis have been described. Indolent olecranon bursitis can be tender. Sometimes there is systemic involvement including cholangitis, chronic meningitis and retinitis. There have been cases of peritonitis after peritoneal dialysis.

Diagnosis is made by biopsy. The pathogens are morphologically similar to mulberries. Confusion with yeasts is possible. For tissue sections a PAS [periodic acid-Schiff] or a Gomori methenamine silver stain are used.

Treatment is surgical with or without amphotericin B. Ketoconazole has frequently been used with success, but requires long-term administration. The possible therapeutic roles of itraconazole and fluconazole need to be better determined.

## Babesiosis

### General

Babesiosis is a zoonotic disease which is triggered by infection with a protozoon of the genus *Babesia*. The disease is also known as piroplasmosis. The order of Piroplasmida belongs to the Apicomplexa (cf. malaria). There are more than 110 species in the genus *Babesia*. Some infect fish, birds, reptiles or mammals. The rodent parasite *Babesia microti* (USA) and the bovine parasites *B. divergens* and *B. bovis* (Europe) cause most infections in humans. Occasionally other species may be responsible for human infections (e.g. the WA1 strain = *B. duncani*).

### Transmission

Voles form the reservoir. Transmission is via the bite of hard ticks such as *Ixodes scapularis* and *Ixodes ricinus*. In the USA larval nymphs of *Ixodes scapularis* feed chiefly on *Peromyscus maniculatus* (“the white-footed deer mouse”). The adult ticks suck blood from deer (cf. Lyme disease). Strangely enough the deer are not infected with *B. microti*. In ticks trans-stadial transmission occurs. The parasite passes from larva to nymph to adult tick. There is no transovarian transmission. Infections in humans are accidental occurrences. After injection of saliva of the tick, the micro-organisms penetrate red blood cells and mature. *Babesia microti* trophozoites undergo asexual reproduction in human blood and divide into two or four merozoites. Infected red blood cells undergo haemolysis. This releases the protista which can then penetrate new red blood cells. Infections via blood transfusions...
have been described. Transplacental infection may occur.

**Geographical distribution**

Endemic regions in the USA include Massachusetts and New York State with Nantucket Island, Long Island, the coast of Connecticut as well as foci in Georgia, California and Wisconsin. Cases have also been reported from various European countries such as Ireland, Scotland, Sweden, former Yugoslavia, France and Russia. There have been isolated case reports from Africa, Asia and Latin America.

**Clinical aspects**

Asymptomatic infection may persist for months or years. If symptomatic, the first symptoms occur after an incubation period of one to two weeks. Malaise, tiredness, fever, headache, nausea and abdominal pain, myalgia and joint pain are early but non-specific symptoms. The body temperature may rise to 40°C. Hepatosplenomegaly with haemolysis and jaundice, haemoglobinuria, mild neutropenia and thrombocytopenia follow. In severe cases ARDS [acute respiratory distress syndrome] with shock may develop. Infections may have a dramatic course in asplenic persons, chiefly in the European forms.

**Diagnosis**
Diagnosis is made from a blood smear stained with Giemsa. The parasitaemia is generally 1 to 10%. Sometimes the mature parasite is in the form of a clover leaf: a so-called tetrad or Maltese cross. The intra-erythrocytic dimension of the merozoite is 1 to 2.5 µm. It is pear-shaped, oval or round. The circular appearance means that Babesia is often confused with Plasmodium falciparum, but malaria pigment cannot be detected. There are also no gametocytes or malaria schizonts. In Babesia infections, large parasites may contain a central white vacuole, which is not present in malaria. Serological tests and DNA analysis may help in diagnosis.

**Treatment**

Quinine is the drug of choice, 650 mg TDS plus clindamycin 600 mg TDS or 1.2g BD IV for 7 to 10 days. Children receive 25 mg quinine/kg/day. Atovaquone (750 mg BD) and azithromycin (500 mg on day 1, then 250 mg daily) are also used and this combination is better tolerated. Exchange
transfusion may be considered if there is life-threatening parasitaemia. A blood transfusion may be life-saving. Remember that ticks can be infected with more than one pathogen. In endemic regions co-infection with *Borrelia burgdorferi*, certain *Ricketssia, Anaplasma, Ehrlichia* or viral pathogen must be considered.

**Prevention**

Asplenic persons should avoid endemic regions and pay extra attention to tick prevention (proper clothes, repellent containing at least 30% DEET, permethrine and physical inspection after walking).

**Free-living amoebae**

Saprophytic amoebae from water, silt and wet soil which belong to the genera *Naegleria, Acanthamoeba, Balamuthia* and *Sappinia* are cosmopolitan and potentially pathogenic. They seldom cause infection although underreporting is probable. In industrialised countries with a moderate climate these amoebae prefer fresh water with a temperature higher than average, such as public swimming pools and warm waste water from factories or power stations. This suggests that these amoebae must be widely distributed in a tropical environment.

**Naegleria fowleri**
Naegleria fowleri trophozoite, one of the free-living amoebae.
Naegleria fowleri amoebae. Copyright ITM
Acanthamoeba sp. Notice the typical thorn-like projections (acanthopoda). Copyright ITM

**Historical note**

Culberston et al in 1958 were the first to launch the concept that free-living soil and water amoebae could cause disease in humans. In order to feed trophozoites form a kind of “cell-mouth”, called an amoebostome. This is quite spectacular in electron microscopic pictures. In addition to phagocytosis via these food cups, contact-mediated cytolysis occurs. When flagella develop, e.g. after transfer from culture or from tissue to water, they sprout at the broad blunt end. This change to the flagellate form can take 2-20 hours. The flagellate form does not divide, but is motile. When the flagella are lost, the amoeboid form is regained and the parasite resumes asexual reproduction. Cysts measure 7-15 µm, but are absent from human tissue, in contrast with *Acanthamoeba* infections.
Infection with *Naegleria fowleri* is the consequence of bathing or swimming in contaminated freshwater ponds or lakes at quite high temperatures, such as fresh water lakes in the summer (e.g. southern USA) ponds, rivers and hot springs. Sampling of such warm water has indicated that *N. fowleri* is commonly present in such environments. The infection follows penetration of water into the nasal cavities. From there the lamina cribiforma of the ethmoid bone is penetrated, probably through phagocytosis of the olfactory epithelium. Via the first cranial nerve, the infection spreads to the lowermost part of the frontal cerebral lobes. Extensive tissue damage follows. The amoebae reproduce rapidly in the cerebrospinal fluid. There is virtually no inflammatory reaction. Haemorrhagic necrosis of the base of the brain, cerebral cortex and the olfactory lobes develops.

The incubation time is 2 to 15 days. Early in the infection, upper respiratory distress, severe headache, sore throat, runny or stuffy nose, altered smell and taste occur. Fever, vomiting and neck stiffness follow. Mental confusion and coma occur after 3 to 6 days. Most infections are lethal. A high index of suspicion is needed for diagnosis since CSF findings are very similar in acute bacterial meningitis. A history of bathing in surface water during the previous two weeks is significant. The disease closely resembles acute bacterial meningitis and is known as primary amoebic encephalitis (PAM).

In clinical practice, most cases will be diagnosed only at autopsy (immunofluorescence and immunoperoxidase techniques).

Immediate chemotherapy is required for survival. *N. fowleri* is sensitive to amphotericin B. Combination treatment with ampho B, miltefosine, miconazole, rifampicin, azithromycin, chloramphenicol and/or ketoconazole has been used. Specialised advice is absolutely required.

**Balamuthia mandrillaris**
Balamuthia mandrillaris infection with important skin lesion. Copyright Alexander von Humboldt Institute, Peru

It is not clear how humans get infected, but transmission via swimming in contaminated surface water is one possibility. The pathogen has also been isolated from a potted plant in a home. Infection with this amoeba causes peri-orbital swelling and ulceration, followed by symptoms of granulomatous meningo-encephalitis, in both immunocompetent and immunocompromised persons. Symptoms include headache, nausea, vomiting, fever, visual disturbances, dysphagia, seizures and hemiparesis. Both trophozoites and cysts are found in CNS tissues. Differentiation with Acanthamoeba is difficult when using only simple light microscopy. Electron microscopy, immunofluorescence testing and histochemistry are needed for definite species identification. In vitro studies show that B. mandrillaris is susceptible to pentamidine. Ketoconazole, propamidine, miltefosine, 5-flucytosine, clotrimazole, sulfadiazine, fluconazole and clarithromycine have all been used in treatment of patients. Treatment is not standardised yet. Experience in Peru has shown that prolonged administration of itraconazole...
400 mg/day (adults) can be useful.

**Acanthamoeba sp.**

Acanthamoeba sp are free-living protista which occur in numerous places (water, dust, waste). Several species have been described: A. castellani, A. culbertsoni, A. polyphaga, A. healyi, A. astronyxis, A. hatchetti, A. rhysodes, A. griffini, A. quina, A. lugdunensis.

Acanthamoeba species are responsible for several clinical problems: (1) granulomatous amoebic encephalitis, (2) keratitis, (3) disseminated lesions, including skin ulcers, but also lesions in adrenals, kidneys, liver, spleen, thyroid....

1. **Granulomatous amoebic encephalitis (GAE):** unlike with Naegleria, infection of the central nervous system progresses slowly and occurs where there is immunosuppression or in the course of a severe general illness. Infections are more common in AIDS patients with a low CD4-count. Generally it presents as a subacute meningo-encephalitis with signs of a brain abscess and develops in two to three weeks (range 7 days – 5 months). The cerebral hemispheres tend to be involved with an inflammatory exudate covering the cortex, granulomatous necrosis of the brain parenchyma and thrombosed blood vessels. Such infections can mimic malignancies, fungal infections or abscesses. In AIDS patients, the differential diagnosis with cerebral toxoplasmosis can be very difficult.

2. **Keratitis.** This is more common than cerebral inflammation. The amoebae may infect small wounds of the cornea and then trigger a dangerous ulcerative keratitis which may develop into painful uveitis with hypopyon, scleritis and panophthalmitis. Acanthamoeba keratitis should be considered in the differential diagnosis of uveitis in AIDS patients. Initially this diagnosis is often missed and the lesion is considered to be a herpetic or fungal keratitis. Infection can follow corneal trauma (e.g. corpus alienum). The number of cases has grown in recent years as the result of increased use of contact lenses and the practice of rinsing these with tap water, as a result this is a cosmopolitan infection. It is likely that bacteria in the biofilm on dirty contact lenses constitute a good source of nutrition for the amoebae. The amoebae are often scarce in corneal smears. Culture is possible on nonnutrient agar plates with an overlay growth of Esch. coli or Pseudomonas aeruginosa bacteria on which the trophozoites feed. Sometimes the diagnosis is made purely on anatomopathological grounds, e.g. during a cornea transplantation.

3. **Other locations.** Abscesses in other locations and granulomatous skin lesions in which histological investigations show amoebae, have also been observed. Skin lesions are more common in AIDS patients. Hard erythematous papulonodular lesions or non-healing indurated ulcers may be the
first sign.

The optimal approach for GAE management is uncertain; therefore, combination regimens are preferred over single-drug regimens. An empiric treatment could be a combination of miltefosine, fluconazole, and pentamidine isethionate. Trimethoprim-sulfamethoxazole, metronidazole and a macrolide (azithromycin or clarithromycin) can be added to this regimen as well. Single cerebral lesions should be resected if possible.

Treatment of Acanthamoeba keratitis employs a combination of propamidine isethionate eye drops (Brolene®), topical neomycin, polyhexamethylene biguanide collyre (Lavasept®) and/or topical chlorhexidine (Hibitane®). Brolene® available in Great Britain is an antiseptic which is moderately toxic for the corneal epithelium. The use of topical steroids is controversial but probably beneficial. Oral itraconazole is probably also active. Topical miconazole is sometimes also used. Pentamidine (a diamidine related to propamidine) is being evaluated. Chronic refractory cases may require corneal transplantation. Unresponsive cases may require enucleation.

<table>
<thead>
<tr>
<th>COMPARISON</th>
<th>Naegleria fowleri</th>
<th>Acanthamoeba sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoebic form with lobate pseudopodia; Flagellate form (two flagella)</td>
<td>No flagella, filiform sharp pseudopodia</td>
<td></td>
</tr>
<tr>
<td>Cysts not present in tissue; they are small and smooth</td>
<td>Cysts can be found in tissues; large and wrinkled with a double wall</td>
<td></td>
</tr>
<tr>
<td>Culture requires living cells (bacteria or cell culture) No growth if NaCl concentration &gt; 0.4%</td>
<td>May grow without bacteria; not affected by NaCl 0.85%</td>
<td></td>
</tr>
<tr>
<td>Smaller than Acanthamoeba; dense endoplasm; less distinct nuclear staining</td>
<td>Large round, less endoplasm; more distinct nucleus</td>
<td></td>
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