Insect-Borne Diseases

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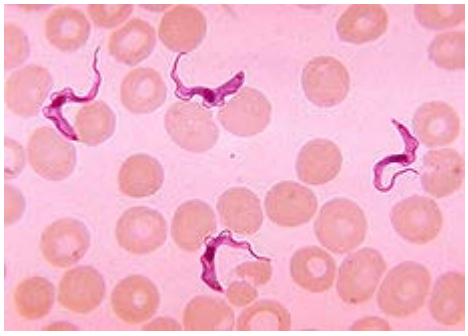
Table of Contents

Chapter 1

African Trypanosomiasis

African trypanosomiasis



Trypanosoma forms in a blood smear.

ICD-10	B56.
ICD-9	086.5
DiseasesDB	29277 13400
MedlinePlus	001362
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MeSH	D014353

Human African trypanosomiasis, **sleeping sickness**, **African lethargy**, or **Congo trypanosomiasis** is a parasitic disease of people and animals, caused by protozoa of the species *Trypanosoma brucei* and transmitted by the tsetse fly. The disease is endemic in some regions of sub-Saharan Africa, covering about 36 countries and 60 million people. It is estimated that 50,000 to 70,000 people are currently infected, the number having declined somewhat in recent years. It is believed that many cases go unreported. About 48,000 people died of it in 2008. Four major epidemics have occurred in recent history: one from 1896–1906 primarily in Uganda and the Congo Basin, two epidemics in 1920 and 1970 in several African countries, and a recent 2008 epidemic in Uganda.

Signs and symptoms

African trypanosomiasis symptoms occur in two stages. The first stage is known as the haemolymphatic phase and is characterized by fever, headaches, joint pains, and itching. Invasion of the circulatory and lymphatic system by the parasites is associated with severe swelling of lymph nodes, often to tremendous sizes. Winterbottom's sign, the tell-tale swollen lymph nodes along the back of the neck, may appear. If left untreated, the disease overcomes the host's defences and can cause more extensive damage, broadening symptoms to include anemia, endocrine, cardiac, and kidney dysfunctions. The second stage, called the neurological phase, begins when the parasite invades the central nervous system by passing through the blood-brain barrier. The term 'sleeping sickness' comes from the symptoms of the neurological phase. The symptoms include confusion, reduced coordination, and disruption of the sleep cycle, with bouts of fatigue punctuated with manic periods leading to daytime slumber and night-time insomnia. Without treatment, the disease is invariably fatal, with progressive mental deterioration leading to coma and death. Damage caused in the neurological phase is irreversible.

In addition to the bite of the tsetse fly, the disease can be transmitted in the following ways:

- Mother to child infection: the trypanosome can sometimes cross the placenta and infect the fetus.
- Laboratories: accidental infections, for example, through the handling of blood of an infected person and organ transplantation, although this is uncommon.
- Blood transfusion
- Sexual contact (might be possible, but happens rarely)

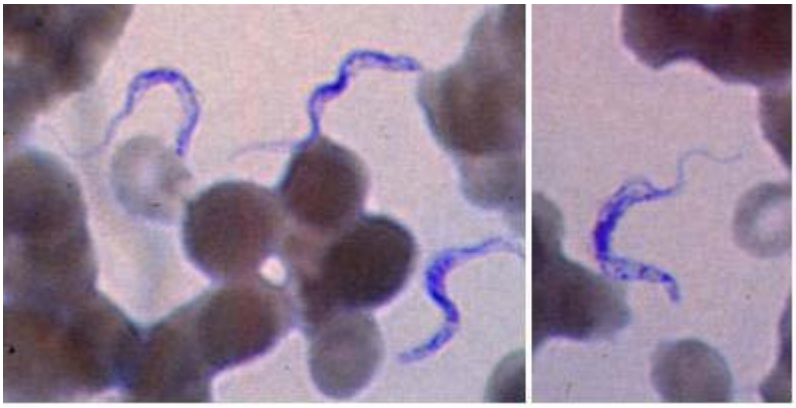
Life cycle

The tsetse fly (genus *Glossina*) is a large, brown biting fly that serves as both a host and vector for the Trypanosome parasites. While taking blood from a mammalian host, an infected tsetse fly injects metacyclic trypomastigotes into skin tissue. From the bite, parasites first enter the lymphatic system and then pass into the bloodstream. Inside the mammalian host, they transform into bloodstream trypomastigotes, and are carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue to replicate by binary fission.

The entire life cycle of African trypanosomes is represented by extracellular stages. A tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host. In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission.

The entire life cycle in the fly takes approximately 3 weeks.

Diagnosis



Two areas from a blood smear from a patient with African trypanosomiasis. Thin blood smear stained with Giemsa. Typical trypomastigote stages (the only stages found in patients), with a posterior kinetoplast, a centrally located nucleus, an undulating membrane, and an anterior flagellum. The two Trypanosoma brucei subspecies that cause human trypanosomiasis, T. b. gambiense and T. b. rhodesiense, are indistinguishable morphologically. The trypanosomes length range is 14 to 33 μm, Source: CDC

The gold standard for diagnosis is identification of trypanosomes in a patient sample by microscopic examination. Patient samples that can be used for diagnosis include chancre fluid, lymph node aspirates, blood, bone marrow, and, during the neurological stage, cerebrospinal fluid. Detection of trypanosome-specific antibodies can be used for diagnosis, but the sensitivity and specificity of these methods are too variable to be used alone for clinical diagnosis. Further, seroconversion occurs after the onset of clinical symptoms during a *T. b. rhodesiense* infection, and therefore is of limited diagnostic use.

Trypanosomes can be detected from patient samples using two different preparations. A wet preparation can be used to look for the motile trypanosomes. Alternatively, a fixed (dried) smear can be stained with Giemsa (or Field) and examined. Often the parasite is in relatively low abundance in the sample, so techniques to concentrate the parasites can be used prior to microscopic examination. For blood samples, these include centrifugation followed by examination of the buffy coat; mini anion-exchange/centrifugation; and the Quantitative Buffy Coat (QBC) technique. For other samples such as spinal fluid, concentration techniques include centrifugation followed by examination of the sediment.

Three serological tests are also available for detection of the parasite: the micro-CATT, wb-CATT, and wb-LATEX. The first uses dried blood while the other two use whole blood samples. A 2002 study found the wb-CATT to be the most efficient for diagnosis, while the wb-LATEX is a better exam for situations where greater sensitivity is required.

Prevention

Two alternative strategies have been used in the attempts to reduce the African trypanosomiases. The primary method focuses on the eradication of the tsetse fly, which disrupts transmission rates by reducing the number of flies. Instances of sleeping sickness are being reduced by the use of the sterile insect technique. The second tactic is primarily medical or veterinary and tries to reduce spread of the parasite by monitoring, prophylaxis, treatment, and surveillance to reduce the number of people/animals that carry the disease.

Regular active surveillance, involving detection and prompt treatment of new infections, and tsetse fly control is the backbone of the strategy used to control sleeping sickness. Systematic screening of at-risk communities is the best approach, because case-by-case screening is not practical in endemic regions. Systematic screening may be in the form of mobile clinics or fixed screening centres where teams travel daily to areas of high infection rates. Such screening efforts are important because early sympotoms are not evident or serious enough to warrant patients with gambiense disease to seek medical attention, particularly in very remote areas. Also, diagnosis of the disease is difficult and health workers may not associate such general symptoms with trypanosomiasis. Systematic screening allows early-stage disease to be detected and treated before the disease progresses, and removes the potential human reservoir. There is a single case report of sexual transmission of West African sleeping sickness, but this is not believed to be an important route of transmission.

Treatment

First line, first stage

The current standard treatment for first stage (haemolymphatic) disease is:

- Intravenous or intramuscular pentamidine (for *T.b. gambiense*); or
- Intravenous suramin (for *T.b. rhodesiense*)

The drug Eflornithine — previously used only as an alternative treatment for sleeping sickness due to its labour-intensive administration — was found to be safe and effective as a first-line treatment for the disease in 2008, according to the Science and Development Network's Sub-Saharan Africa news updates. . Researchers tracked over 1,000 adults and children at a centre in Ibba, Southern Sudan—the first use of eflornithine on a large scale— and it was highly effective in treating the issue.

According to a treatment study of Trypanosoma gambiense caused human African trypanosomiasis, use of eflornithine (DMFO) resulted in fewer adverse events than treatment with melarsoprol.

First line, second stage

The current standard treatment for second stage (neurological phase) disease is:

- Intravenous melarsoprol 2.2 mg/kg daily for 12 consecutive days.

Alternative first line therapies include:

- Intravenous melarsoprol 0.6 mg/kg on day 1, 1.2 mg/kg IV melarsoprol on day 2, and 1.2 mg/kg/day IV melarsoprol combined with oral 7.5 mg/kg nifurtimox twice a day on days 3 to 10; or
- Intravenous eflornithine 50 mg/kg every six hours for 14 days.

Combination therapy with eflornithine and nifurtimox is safer and easier than treatment with eflornithine alone, and appears to be equally or more effective. It has been recommended as first-line treatment for second stage *T. b. gambiensis* disease.

Resistant disease

In areas with melarsoprol resistance or in patients who have relapsed after melarsoprol monotherapy , the treatment should be:

- melarsoprol and nifurtimox, or
- eflornithine

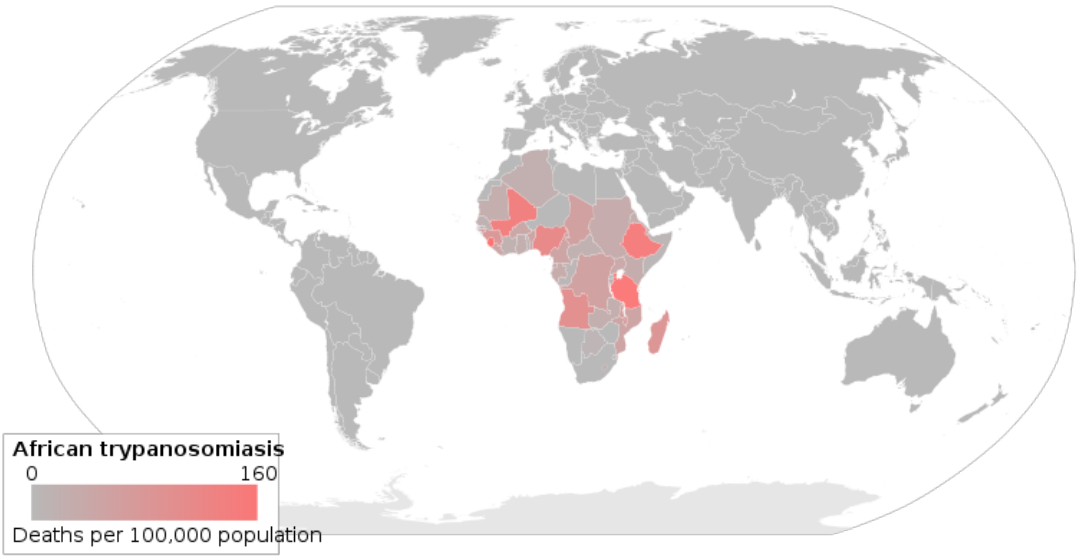
Outdated protocols

The following traditional regimens should no longer be used:

- (old "standard" 26-day melarsoprol therapy) Intravenous melarsoprol therapy (3 series of 3.6 mg/kg/day intravenously for 3 days, with 7-day breaks between the series) (this regimen is less convenient and patients are less likely to complete therapy);
- (incremental melarsoprol therapy) 10-day incremental-dose melarsoprol therapy (0.6 mg/kg iv on day 1, 1.2 mg/kg iv on day 2, and 1.8 mg/kg iv on days 3–10) (previously thought to reduce the risk of treatment-induced encephalopathy, but now known to be associated with an increased risk of relapse and a higher incidence of encephalopathy);

After successful treatment, all patients should be followed up for two years with lumbar punctures every six months to look for relapse.

Epidemiology



Deaths per 100,000 population due to African trypanosomiasis by country in 2002

The disease is found in two forms, depending on the parasite, either *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense*. Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in pigs and other animals. Wild game animals and cattle are the main reservoir of *T. b. rhodesiense*. *T. b. gambiense* is found in central and western Africa; it causes a chronic condition that can remain in a passive phase for months or years before symptoms emerge. *T. b. rhodesiense* is the acute form of the disease, but has a much more limited geographic range. It is found in southern and eastern Africa and symptoms of infection emerges in a few weeks and is more virulent and faster developing than *T. b. gambiense*. According to recent estimates, the disability adjusted life years (9 to 10 years) (DALYs) lost due to sleeping sickness are 2.0 million. Recent estimates indicate that over 60 million people living in some 250 locations are at risk of contracting the disease, and there were under 10,000 cases reported in 2009 according to WHO figures which represents a huge decrease from the estimated 300,000 new cases in 1998. The disease has been recorded as occurring in 36 countries, all in sub-Saharan Africa. It is endemic in southeast Uganda and western Kenya, and kills more than 48,000 Africans in 2008.

Horse-flies (Tabanidae) and stable flies (Muscidae) possibly play a role in transmission of nagana (the animal form of sleeping sickness) and the human disease form.

History

The condition has been present in Africa since at least the 14th century, and probably for thousands of years before that. Because there was a lack of travel between indigeneous people, sleeping sickness in humans had been limited to isolated pockets. This changed once Henry Morton Stanley had explored the Congo basin as in his wake explorers,

traders, and slavers arrived bringing parasites along. Gambian sleeping sickness travelled up the Congo River, then further eastwards. In 1901 a devastating epidemic had erupted in Uganda, killing more than 250,000 people, about two-thirds of the population in the affected lake-shore areas. It has been estimated that up to half the people died of sleeping sickness and smallpox in the lands along the lower Congo River.

The causative agent and vector were identified in 1902–1903 by Sir David Bruce, and the differentiation between the subspecies of the protozoa made in 1910. The first effective treatment, atoxyl, an arsenic-based drug developed by Paul Ehrlich and Kiyoshi Shiga, was introduced in 1910, but blindness was a serious side effect. Numerous drugs designed to treat the disease have been introduced since then.

Suramin was introduced in 1920 to treat the first stage of the disease. By 1922, Suramin was generally combined with Tryparsamide (another pentavalent organo-arsenic drug) in the treatment of the second stage of the gambiense form. It was used during the grand epidemic in West and Central Africa in millions of people and was the mainstay of therapy until 1969.

Pentamidine, a highly effective drug for the first stage of the disease, has been used since 1939. During the fifties, it was widely used as a prophylactic agent in Western Africa, leading to a sharp decline in infection rates. At the time, it was thought that eradication of the disease was at hand.

The organo-arsenical melarsoprol (Arsobal) was developed in the 1940s, and is effective for patients with second stage sleeping sickness. However, 3-10% of those injected have reactive encephalopathy (convulsions, progressive coma, or psychotic reactions), and 10-70% of such cases result in death; it can cause brain damage in those who survive the encephalopathy. However, due to its effectiveness, melarsoprol is still used today. Resistance to melarsoprol is increasing, and combination therapy with nifurtimox is currently under research.

Eflornithine (difluoromethylornithine or DFMO), the most modern treatment, was developed in the 1970s by Albert Sjoerdsmanot and underwent clinical trials in the 1980s. The drug was approved by the United States Food and Drug Administration in 1990, but Aventis, the company responsible for its manufacture, halted production in 1999. In 2001, however, Aventis, in association with Médecins Sans Frontières and the World Health Organization, signed a long-term agreement to manufacture and donate the drug.

Research

The genome of the parasite has been sequenced and several proteins have been identified as potential targets for drug treatment. Analysis of the genome also revealed the reason why generating a vaccine for this disease has been so difficult. *T. brucei* has over 800 genes that make proteins the parasite "mixes and matches" to evade immune system detection.

Recent findings indicate that the parasite is unable to survive in the bloodstream without its flagellum. This insight gives researchers a new angle with which to attack the parasite.

A new treatment based on a truncated version of the apolipoprotein L-1 of high density lipoprotein and a single-domain antibody has recently been found to work in mice, but has not been tested in humans.

The cover story of the August 25, 2006 issue of the journal Cell describes an advance in understanding how Trypanosomes escape the immune system. Dr. Lee Soo Hee and colleagues, working at Johns Hopkins investigated the pathway by which Trypanosomes make myristate, a 14-carbon length fatty acid. Myristate is a component of the variant surface glycoprotein (VSG), the molecule that makes up the trypanosome's outer layer. This outer surface coat of VSG is vital to the trypanosome's ability to avoid destruction by the host's immune system. Dr. Lee and colleagues discovered trypanosomes use a novel fatty acid synthesis pathway involving fatty acid elongases to make myristate and other fatty acids.

An international research team working in the Democratic Republic of the Congo, Southern Sudan, and Angola involving Immtech International and University of North Carolina at Chapel Hill have completed a Phase IIb clinical trial and began a Phase III trial in 2005 testing the efficacy of the first oral treatment for Sleeping Sickness, known at this point as "DB289".

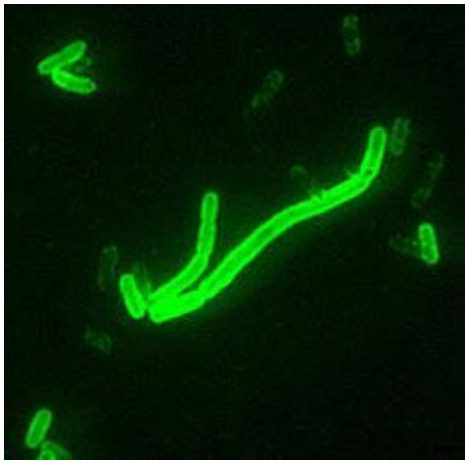
Trypanosomiasis vaccines are undergoing research.

Two independent variants of the APOL1 gene found in African haplotypes carrying signatures of natural selection have been shown to confer protection against the acute version of sleeping sickness caused by *Trypanosoma brucei rhodesiense* while at the same time increasing risk of kidney disease when inherited from both parents.

Chapter 2

Plague

Plague



Yersinia pestis seen at 200× magnification with a fluorescent label. This bacterium, carried and spread by fleas, is the cause of the various forms of the disease plague.

ICD-10	A20.
ICD-9	020
MedlinePlus	000596
eMedicine	med/3381
MeSH	D010930

Plague is one of three epidemic diseases still subject to the International Health Regulations and notifiable to the World Health Organization (such as tuberculosis or typhus). The deadly infectious disease is caused by the enterobacteria *Yersinia pestis*, named after the French bacteriologist A.J.E. Yersin. Primarily carried by rodents (most notably rats) and spread to humans via fleas, the disease is notorious throughout history, due to the unrivaled scale of death and devastation it brought. Depending on lung infection, or sanitary conditions, plague also can be spread in the air, by direct contact, or by contaminated undercooked food or materials. The symptoms of plague depend on the

concentrated areas of infection in each person: such as bubonic plague in lymph nodes, septicemic plague in blood vessels, pneumonic plague in lungs, etc. Medicines can cure plague if detected early. Plague is still endemic in some parts of the world.

Name

The epidemiological use of the term *"plague"* is currently applied to bacterial infections that cause *buboes*, although historically the medical use of the term "plague" has been applied to pandemic infections in general. Plague is often synonymous with "bubonic plague" but this only describes one of its manifestations. Other names have been used to describe this disease, such as "The Black Plague" and "The Black Death"; the latter is now used primarily to describe the second, and most devastating, pandemic of the disease.

The etymology of the word *"plague"* is believed to come from the Latin word *plāga* ("blow, wound") and *plangere* (“to strike, or to strike down”).

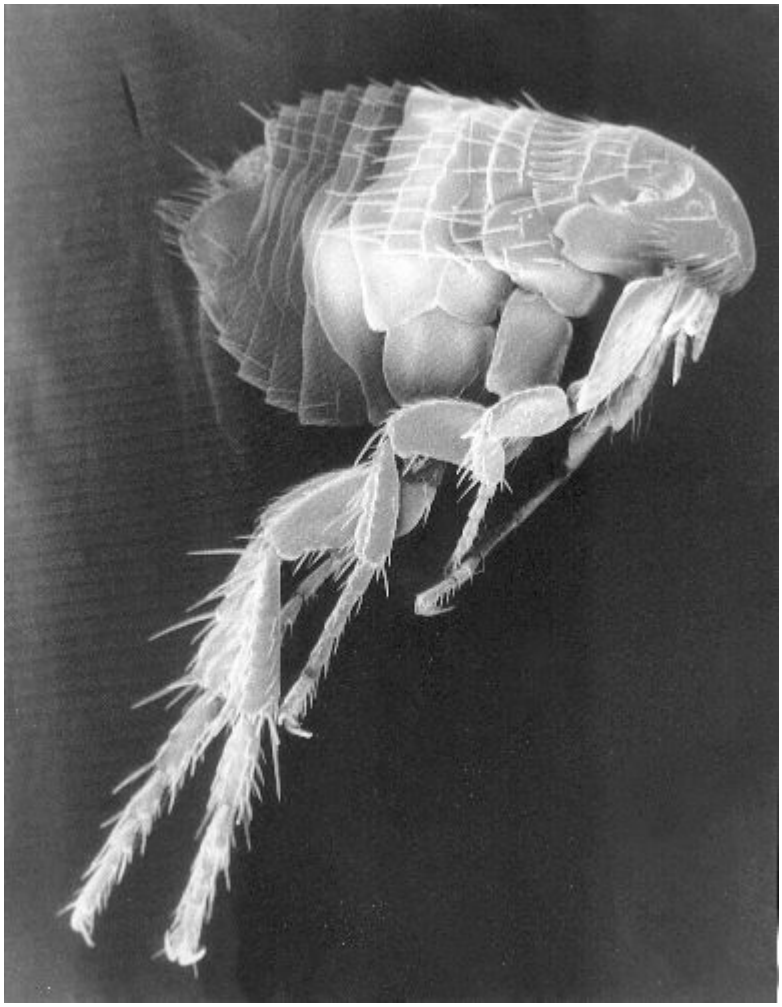
Epidemiology and Distribution of Plague

Infection and transmission

Transmission of *Y. pestis* to an uninfected individual is possible by any of the following means.

- droplet contact - coughing or sneezing on another person
- direct physical contact - touching an infected person, including sexual contact
- indirect contact - usually by touching soil contamination or a contaminated surface
- airborne transmission - if the microorganism can remain in the air for long periods
- fecal-oral transmission - usually from contaminated food or water sources
- vector borne transmission - carried by insects or other animals.

Yersinia pestis circulates in animal reservoirs, particularly in rodents, in the natural foci of infection found on all continents except Australia. The natural foci of plague are situated in a broad belt in the tropical and sub–tropical latitudes and the warmer parts of the temperate latitudes around the globe, between the parallels 55 degrees North and 40 degrees South.



Flea *Xenopsylla cheopis*, primary vector to spread Bubonic plague

Contrary to popular belief, rats did not directly start the spread of the Bubonic plague. It is mainly a disease in the fleas (*Xenopsylla cheopis*) that infested the rats, making the rats themselves the first victims of the plague. Infection in a human occurs when a person is bitten by a flea that has been infected by biting a rodent that itself has been infected by the bite of a flea carrying the disease. The bacteria multiply inside the flea, sticking together to form a plug that blocks its stomach and causes it to starve. The flea then bites a host and continues to feed, even though it cannot quell its hunger, and consequently the flea vomits blood tainted with the bacteria back into the bite wound. The bubonic plague bacterium then infects a new victim, and the flea eventually dies from starvation. Serious outbreaks of plague are usually started by other disease outbreaks in rodents, or a rise in the rodent population.

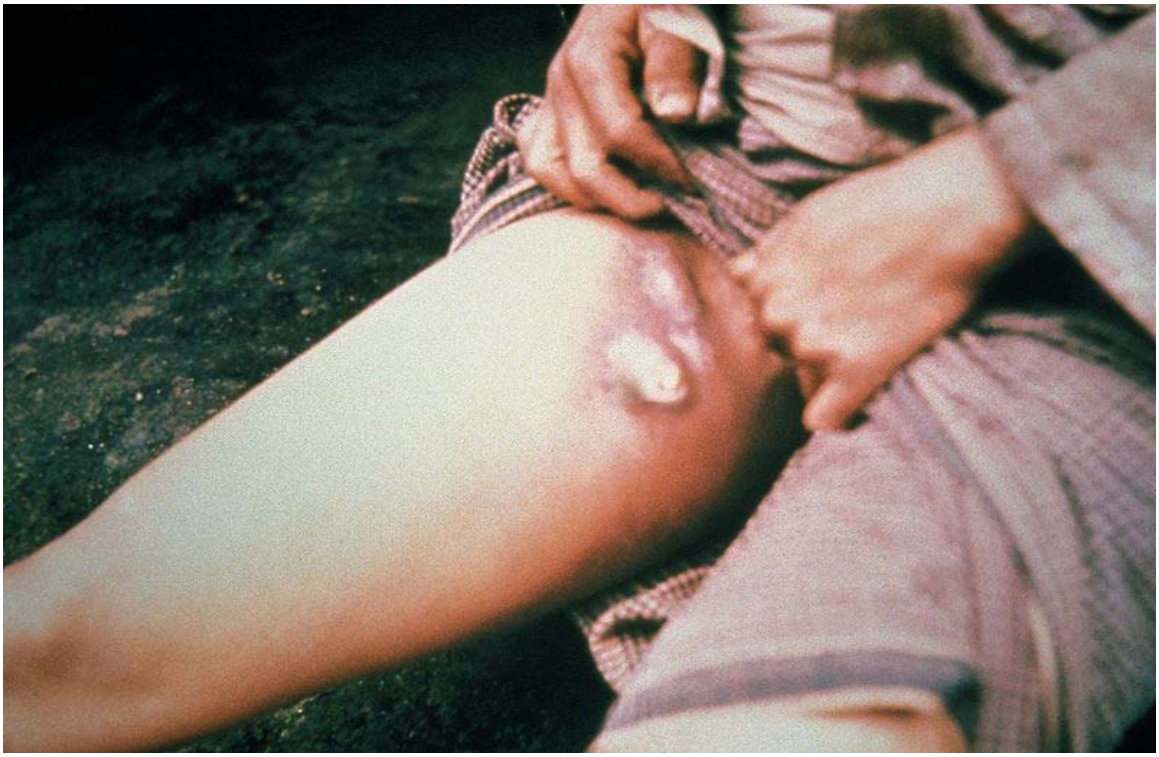
In 1894, two bacteriologists, Alexandre Yersin of France and Kitasato Shibasaburō of Japan, independently isolated the bacterium in Hong Kong responsible for the Third Pandemic. Though both investigators reported their findings, a series of confusing and contradictory statements by Kitasato eventually led to the acceptance of Yersin as the

primary discoverer of the organism. Yersin named it ***Pasteurella pestis*** in honor of the Pasteur Institute, where he worked, but in 1967 it was moved to a new genus, renamed ***Yersinia pestis*** in honor of Yersin. Yersin also noted that rats were affected by plague not only during plague epidemics but also often preceding such epidemics in humans, and that plague was regarded by many locals as a disease of rats: villagers in China and India asserted that, when large numbers of rats were found dead, plague outbreaks soon followed.

In 1898, the French scientist Paul-Louis Simond (who had also come to China to battle the Third Pandemic) established the rat-flea vector that drives the disease. He had noted that persons who became ill did not have to be in close contact with each other to acquire the disease. In Yunnan, China, inhabitants would flee from their homes as soon as they saw dead rats, and on the island of Formosa (Taiwan), residents considered the handling of dead rats heightened the risks of developing plague. These observations led him to suspect that the flea might be an intermediary factor in the transmission of plague, since people acquired plague only if they were in contact with recently dead rats, who had died less than 24 hours before. In a now classic experiment, Simond demonstrated how a healthy rat died of plague, after infected fleas had jumped to it, from a rat which had recently died of the plague.

Pathology

Bubonic plague



Buboes on the thigh of a person suffering from Bubonic plague

When a flea bites a human and contaminates the wound with regurgitated blood, the plague carrying bacteria are passed into the tissue. *Y. pestis* can reproduce inside cells, so even if phagocytosed, they can still survive. Once in the body, the bacteria can enter the lymphatic system, which drains interstitial fluid. Plague bacteria secrete several toxins, one of which is known to cause dangerous beta-adrenergic blockade.

Y. pestis spreads through the lymphatics of the infected human until it reaches a lymph node, where it stimulates severe haemorrhagic inflammation that causes the lymph nodes to expand. The expansion of lymph nodes is the cause of the characteristic "bubo" associated with the disease.

Septicemic plague

Lymphatics ultimately drain into the bloodstream, so the plague bacteria may enter the blood and travel to almost any part of the body. In septicemic plague, bacterial endotoxins cause disseminated intravascular coagulation (DIC), causing tiny clots throughout the body and possibly ischaemic necrosis (tissue death due to lack of circulation/perfusion to that tissue) from the clots. DIC results in depletion of the body's clotting resources, so that it can no longer control bleeding. Consequently, there is bleeding into the skin and other organs, which can cause red and/or black patchy rash and hemoptysis/hematemesis (coughing up/ vomiting of blood). There are bumps on the skin that look somewhat like insect bites; these are usually red, and sometimes white in the center. Untreated, septicemic plague is usually fatal. Early treatment with antibiotics reduces the mortality rate to between 4 and 15 percent. People who die from this form of plague often die on the same day symptoms first appear.

Pneumonic plague

The pneumonic plague infects the lungs, and with that infection comes the possibility of person-to-person transmission through respiratory droplets. The incubation period for pneumonic plague is usually between two and four days, but can be as little as a few hours. The initial symptoms of headache, weakness, and coughing with blood (hemoptysis), or vomiting blood (hematemesis), are indistinguishable from several other respiratory illnesses. Without diagnosis and treatment, the infection can be fatal in one to six days; mortality in untreated cases is approximately 100%.

Other clinical forms

There are a few other rare manifestations of plague, including asymptomatic plague and abortive plague. *Cellulocutaneous plague* sometimes results in infection of the skin and soft tissue, often around the bite site of a flea.

Treatments

Waldemar Haffkine, a doctor of Russian-Jewish origin who worked in Mumbai, India, was the first to invent and test a plague vaccine against bubonic plague, on January 10, 1897.

The traditional treatments are:

- Streptomycin 30 mg/kg IM twice daily for 7 days
- Chloramphenicol 25–30 mg/kg single dose, followed by 12.5–15 mg/kg four times daily
- Tetracycline 2 g single dose, followed by 500 mg four times daily for 7–10 days (not suitable for children)

More recently,

- Gentamicin 2.5 mg/kg IV or IM twice daily for 7 days
- Doxycycline 100 mg (adults) or 2.2 mg/kg (children) orally twice daily have also been shown to be effective.

History

Amulet (800BC-612BC) to ward off plague inscribed with a quotation from the Akkadian Erra Epic.

"Der Doktor Schnabel von Rom" (English: "Doctor Beak of Rome") engraving by Paul Fürst (after J Columbina). The beak is a primitive gas mask worn by physicians, stuffed with substances (such as spices and herbs) thought to ward off the plague.

Nicolas Poussin (1594-1665), French. The Plague of Ashdod, 1630. Oil on canvas, 148 x 198 cm. Musée du Louvre, Paris, France, Giraudon/Bridgeman Art Library.

The earliest account describing a possible plague epidemic is found in I Samuel 5:6 of the Hebrew Bible (Tanakh). In this account, the Philistines of Ashdod were stricken with a plague for the crime of stealing the Ark of the Covenant from the Children of Israel. These events have been dated to approximately the second half of the eleventh century BC. The word "tumors" is used in most English translations to describe the sores that came upon the Philistines. The Hebrew, however, can be interpreted as "swelling in the secret parts". The account indicates that the Philistine city and its political territory were stricken with a "ravaging of mice" and a plague, bringing death to a large segment of the population.

In the second year of the Peloponnesian War (430 BC), Thucydides described an epidemic disease which was said to have begun in Ethiopia, passed through Egypt and Libya, then come to the Greek world. In the Plague of Athens, the city lost possibly one third of its population, including Pericles. Modern historians disagree on whether the plague was a critical factor in the loss of the war. Although this epidemic has long been considered an outbreak of plague, many modern scholars believe that typhus, smallpox, or measles may better fit the surviving descriptions. A recent study of DNA found in the dental pulp of plague victims suggests that typhoid was actually responsible.

In the first century AD, Rufus of Ephesus, a Greek anatomist, refers to an outbreak of plague in Libya, Egypt, and Syria. He records that Alexandrian doctors named Dioscorides and Posidonius described symptoms including acute fever, pain, agitation, and delirium. Buboes—large, hard, and non-suppurating—developed behind the knees, around the elbows, and "in the usual places." The death toll of those infected was very high. Rufus also wrote that similar buboes were reported by a Dionysius Curtus, who may have practiced medicine in Alexandria in the third century BC. If this is correct, the

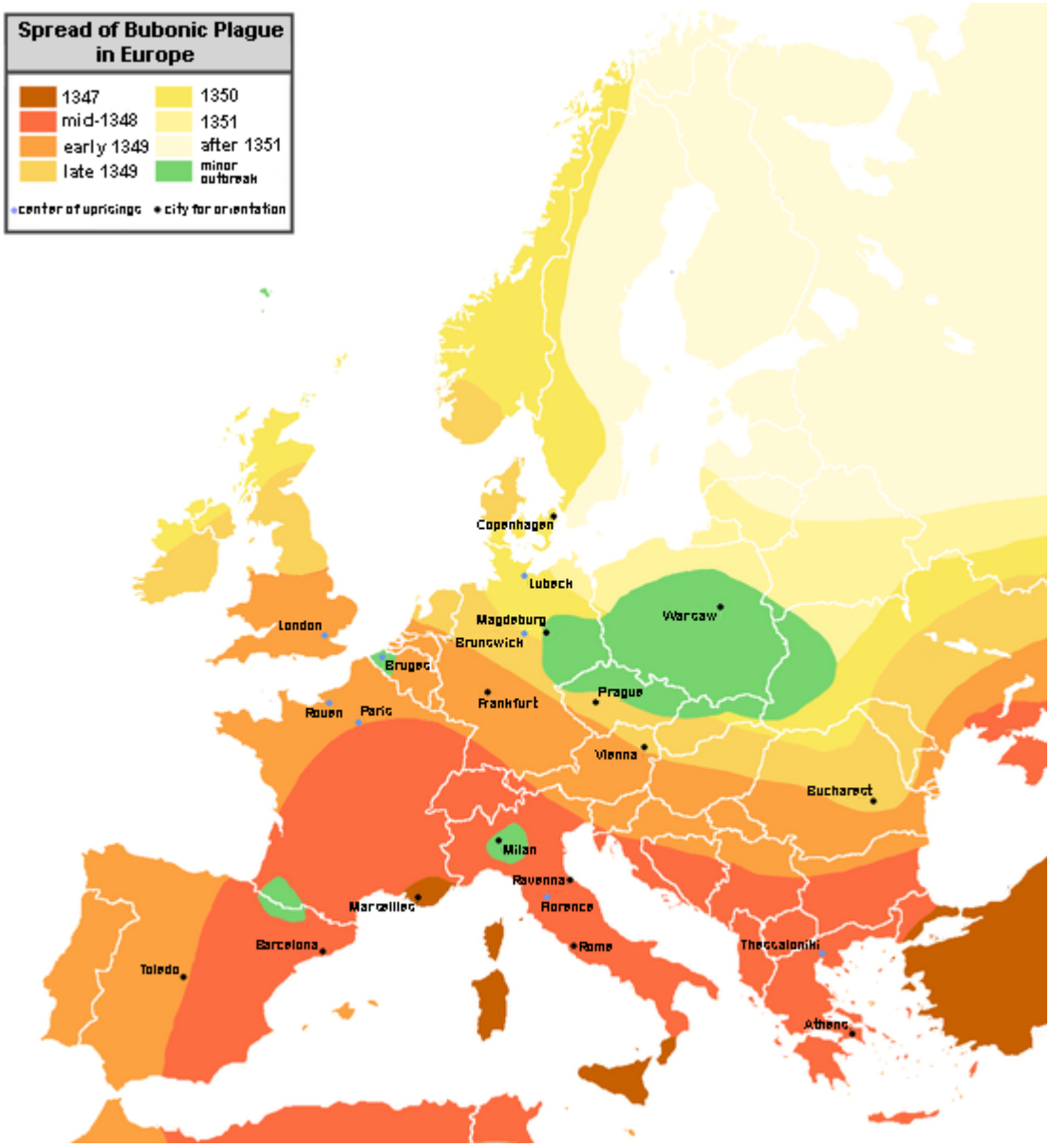
eastern Mediterranean world may have been familiar with bubonic plague at that early date.

First Pandemic: Plague of Justinian

The Plague of Justinian in AD 541–542 is the first known attack on record, and marks the first firmly recorded pattern of bubonic plague. This disease is thought to have originated in China. It then spread to Africa from where the huge city of Constantinople imported massive amounts of grain, mostly from Egypt, to feed its citizens. The grain ships were the source of contagion for the city, with massive public granaries nurturing the rat and flea population. At its peak the plague was killing 10,000 people in Constantinople every day and ultimately destroyed perhaps 40% of the city's inhabitants. It went on to destroy up to a quarter of the human population of the eastern Mediterranean.

In AD 588 a second major wave of plague spread through the Mediterranean into what is now France. It is estimated that the Plague of Justinian killed as many as 100 million people across the world. It caused Europe's population to drop by around 50% between 541 and 700. It also may have contributed to the success of the Arab conquests. An outbreak of it in the AD 560s was described in AD 790 as causing "swellings in the glands...in the manner of a nut or date" in the groin "and in other rather delicate places followed by an unbearable fever". While the swellings in this description have been identified by some as buboes, there is some contention as to whether the pandemic should be attributed to the bubonic plague, *Yersinia pestis*, known in modern times.

Second Pandemic: Black Death



Map showing the spread of bubonic plague in Europe

From 1347 to 1351, the Black Death, a massive and deadly pandemic originating in China, spread along the Silk Road and swept through Asia, Europe and Africa. It may have reduced the world's population from 450 million to between 350 and 375 million. China lost around half of its population, from around 123 million to around 65 million; Europe around 1/3 of its population, from about 75 million to about 50 million; and Africa approximately 1/8th of its population, from around 80 million to 70 million (mortality rates tended to be correlated with population density so Africa, being less dense overall, had the lowest rate). This makes the Black Death the largest death toll from any known non-viral epidemic. Although accurate statistical data does not exist, it is thought that 1.4 million died in England (1/3 of England's 4.2 million people), while an

even higher percentage of Italy's population was likely wiped out. On the other hand, Northeastern Germany, Bohemia, Poland and Hungary are believed to have suffered less, and there are no estimates available for Russia or the Balkans. It is conceivable that Russia may not have been as affected due to its very cold climate and large size, hence often less close contact with the contagion.

The Black Death contributed to the destruction of the feudal system in Medieval Time. As more serfs and workers died, there were fewer people to work for the nobles and they had to give higher wages to the workers willing to work on the nobles' lands. The Black Death also killed many great kings and nobles. In its aftermath, the Black Death may also have favoured the use of more advanced farming tools as a smaller workforce was available and plots grew larger as a result of the population loss.

The plague continued to strike parts of Europe sporadically until the 17th century, each time with reduced intensity and fatality, suggesting an increased resistance due to natural selection. Some have also argued that changes in hygiene habits and efforts to improve public health and sanitation had a significant impact on the falling rates of infection.

Nature of the disease

In the early 20th century, following the identification by Yersin and Kitasato of the plague bacterium that caused the late 19th and early 20th century Asian bubonic plague (the Third Pandemic), most scientists and historians came to believe that the Black Death was an incidence of this plague, with a strong presence of the more contagious pneumonic and septicemic varieties increasing the pace of infection, spreading the disease deep into inland areas of the continents. It was claimed that the disease was spread mainly by black rats in Asia and that therefore there must have been black rats in north-west Europe at the time of the Black Death to spread it, although black rats are currently rare except near the Mediterranean. This led to the development of a theory that brown rats had invaded Europe, largely wiping out black rats, bringing the plagues to an end, although there is no evidence for the theory in historical records. Some historians suggest that marmots, rather than rats, were the primary carriers of the disease.

Many modern researchers have argued that the disease was more likely to have been viral (that is, not bubonic plague), pointing to the absence of rats from some parts of Europe that were badly affected and to the conviction of people at the time that the disease was spread by direct human contact. According to the accounts of the time the black death was extremely virulent, unlike the 19th and early 20th century bubonic plague. Samuel K. Cohn has made a comprehensive attempt to rebut the bubonic plague theory. In the Encyclopedia of Population, he points to five major weaknesses in this theory:

- very different transmission speeds — the Black Death was reported to have spread 385 km in 91 days (4.23 km/day) in 664, compared to 12–15 km a year for the modern Bubonic Plague, with the assistance of trains and cars
- difficulties with the attempt to explain the rapid spread of the Black Death by arguing that it was spread by the rare pneumonic form of the disease — in fact

this form killed less than 0.3% of the infected population in its worst outbreak (Manchuria in 1911)
- different seasonality — the modern plague can only be sustained at temperatures between 10 and 26°C and requires high humidity, while the Black Death occurred even in Norway in the middle of the winter and in the Mediterranean in the middle of hot dry summers
- very different death rates — in several places (including Florence in 1348) over 75% of the population appears to have died; in contrast the highest mortality for the modern Bubonic Plague was 3% in Mumbai in 1903
- the cycles and trends of infection were very different between the diseases — humans did not develop resistance to the modern disease, but resistance to the Black Death rose sharply, so that eventually it became mainly a childhood disease

Cohn also points out that while the identification of the disease as having buboes relies on accounts of Boccaccio and others, they described buboes, abscesses, rashes and carbuncles occurring all over the body, the neck or behind the ears. In contrast, the modern disease rarely has more than one bubo, most commonly in the groin, and is not characterised by abscesses, rashes and carbuncles.

Researchers have offered a mathematical model based on the changing demography of Europe from 1000 to 1800 AD demonstrating how plague epidemics, 1347 to 1670, could have provided the selection pressure that raised the frequency of a mutation to the level seen today that prevent HIV from entering macrophages that carry the mutation (the average frequency of this allele is 10% in European populations). It is suggested that the original single mutation appeared over 2,500 years ago and that persistent epidemics of a haemorrhagic fever struck at the early classical civilizations.

However recent research published in the open-access scientific journal *PloS Pathogens* in October 2010 presented conclusive evidence that two previously unknown clades (variant strains) of *Y. pestis* were responsible for the Black Death. A multinational team conducted new surveys that used both ancient DNA analyses and protein-specific detection to find DNA and protein signatures specific for *Y. pestis* in human skeletons from widely distributed mass graves in northern, central and southern Europe that were associated archaeologically with the Black Death and subsequent resurgences. The authors concluded that this research, together with prior analyses from the south of France and Germany,

"...ends the debate about the etiology of the Black Death, and unambiguously demonstrates that Y. pestis was the causative agent of the epidemic plague that devastated Europe during the Middle Ages."

The study also identified two previously unknown but related strains of *Y. pestis* that were associated with distinct medieval mass graves. These were found to be ancestral to modern isolates of the present-day *Y. pestis* strains 'Orientalis' and 'Medievalis', suggesting that these variant strains (which are now presumed to be extinct) may have entered Europe in two waves. Surveys of plague pit remains in France and England

indicate that the first variant entered Europe through the port of Marseille around November 1347 and spread through France over the next two years, eventually reaching England in the spring of 1349, where it spread through the country in three successive epidemics.

However, surveys of plague pit remains from the Netherlands town of Bergen op Zoom showed evidence of a second *Y. pestis* genotype which differed from that found in Britain and France and this second strain is now thought to have been responsible for the pandemic that spread through the Low Countries from 1350. This discovery implies that Bergen op Zoom (and possibly other parts of the southern Netherlands) was not directly infected from England or France c. AD 1349, and the researchers have suggested that a second wave of plague infection, distinct from that which occurred in Britain and France, may have been carried to the Low Countries from Norway, the Hanseatic cities, or another site.

Third Pandemic

The Third Pandemic began in China in 1855, spreading plague to all inhabited continents and ultimately killing more than 12 million people in India and China alone. Casualty patterns indicate that waves of this pandemic may have come from two different sources. The first was primarily bubonic and was carried around the world through ocean-going trade, transporting infected persons, rats, and cargoes harboring fleas. The second, more virulent strain was primarily pneumonic in character, with a strong person-to-person contagion. This strain was largely confined to Manchuria and Mongolia. Researchers during the "Third Pandemic" identified plague vectors and the plague bacterium, leading in time to modern treatment methods.

Plague occurred in Russia in 1877–1889 in rural areas near the Ural Mountains and the Caspian Sea. Efforts in hygiene and patient isolation reduced the spread of the disease, with approximately 420 deaths in the region. Significantly, the region of Vetlianka in this area is near a population of the bobak marmot, a small rodent considered a very dangerous plague reservoir. The last significant Russian outbreak of Plague was in Siberia in 1910 after sudden demand for Marmot skins (a substitute for Sable) increased the price by 400 percent. The traditional hunters would not hunt a sick Marmot and it was taboo to eat the fat from under the arm (the axillary lymphatic gland that often harboured the plague) so outbreaks tended to be confined to single individuals. The price increase, however, attracted thousands of Chinese hunters from Manchuria who not only caught the sick animals but also ate the fat, which was considered a delicacy. The plague spread from the hunting grounds to the terminus of the Chinese Eastern Railway and then followed the track for 2,700 km. The plague lasted 7 months and killed 60,000 people.

The bubonic plague continued to circulate through different ports globally for the next fifty years; however, it was primarily found in Southeast Asia. An epidemic in Hong Kong in 1894 had particularly high death rates, 90%. As late as 1897, medical authorities in the European powers organized a conference in Venice, seeking ways to keep the plague out of Europe. Mumbai plague epidemic struck the city of Mumbai (Bombay) in

1896. The disease reached the Republic of Hawaii in December 1899, and the Board of Health's decision to initiate controlled burns of select buildings in Honolulu's Chinatown turned into an uncontrolled fire which led to the inadvertent burning of most of Chinatown on January 20, 1900 according to the Star Bulletin's Feature on the Great Chinatown Fire. Plague finally reached the United States later that year in San Francisco, and persisted in Hawaii on the outer islands of Maui and Hawaii (The Big Island) until it was finally eradicated in 1959.

Although the outbreak that began in China in 1855 is conventionally known as the Third Pandemic, it is unclear whether there have been fewer, or more, than three major outbreaks of bubonic plague. Most modern outbreaks of bubonic plague amongst humans have been preceded by a striking, high mortality amongst rats, yet this phenomenon is absent from descriptions of some earlier plagues, especially the Black Death. The buboes, or swellings in the groin, that are especially characteristic of bubonic plague, are a feature of other diseases as well.

Research done by a team of biologists from the Institute of Pasteur in Paris and Johannes Gutenberg University Mainz in Germany by analyzing the DNA and proteins from plague pits was published in Oct., 2010, reported beyond doubt that all 'the three major plagues' were due to at least two previously unknown strains of *Yersinia pestis* and orginated from China Yersinia pestis bacteria clearly identified as the cause of the big plague epidemic of the Middle Ages. A team of medical geneticists led by Mark Achtman of University College Cork in Ireland reconstructed a family tree of the bacterium and concluded in an online issue of Nature Genetics published on 31 Oct., 2010 that all three of the great waves of plague originated from China. Europe's Plagues Came From China, Study Finds.

Plague as a biological weapon

Plague has a long history as a biological weapon. Historical accounts from ancient China and medieval Europe detail the use of infected animal carcasses, such as cows or horses, and human carcasses, by the Xiongnu/Huns, Mongols, Turks, and other groups, to contaminate enemy water supplies. Han Dynasty General Huo Qubing is recorded to have died of such a contamination while engaging in warfare against the Xiongnu. Plague victims were also reported to have been tossed by catapult into cities under siege.

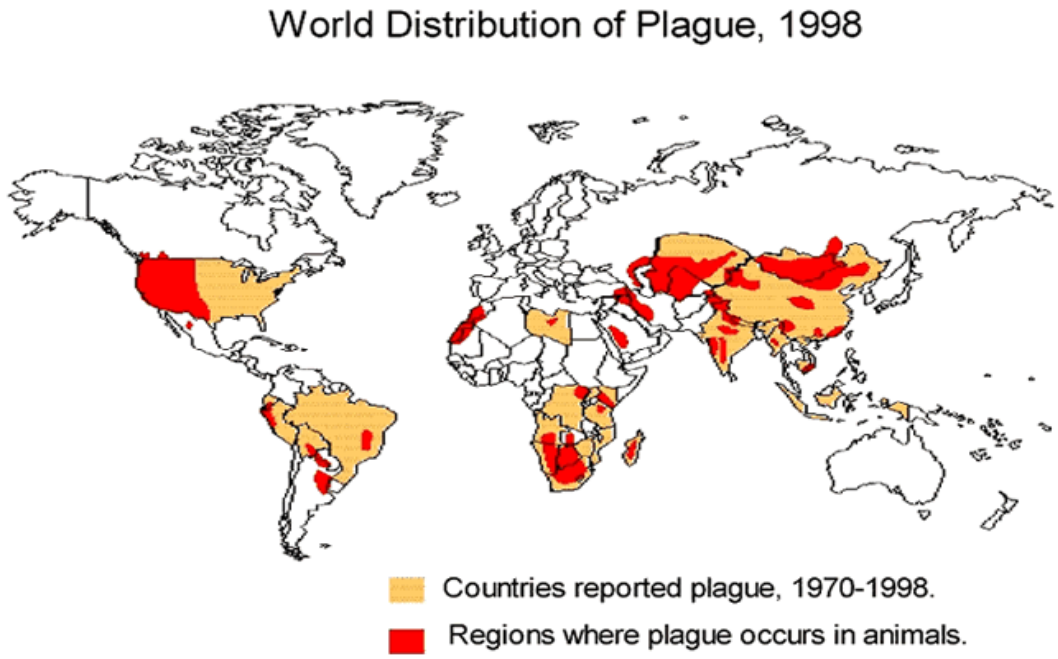
In 1347, the Genoese possession of Caffa, a great trade emporium on the Crimean peninsula, came under siege by an army of Mongol warriors of the Golden Horde under the command of Janibeg. After a protracted siege during which the Mongol army was reportedly withering from the disease, they decided to use the infected corpses as a biological weapon. The corpses were catapulted over the city walls, infecting the inhabitants. The Genoese traders fled, transferring the plague (Black Death) via their ships into the south of Europe, whence it rapidly spread.

During World War II, the Japanese Army developed weaponised plague, based on the breeding and release of large numbers of fleas. During the Japanese occupation of

Manchuria, Unit 731 deliberately infected Chinese, Korean, and Manchurian civilians and prisoners of war with the plague bacterium. These subjects, termed "maruta", or "logs", were then studied by dissection, others by vivisection while still conscious. Members of the unit such as Shiro Ishii were exonerated from the Tokyo tribunal by Douglas MacArthur but twelve of them were prosecuted in the Khabarovsk War Crime Trials in 1949 during which some admitted having spread Bubonic plague within a 36-km radius around the city of Changde.

Ishii innovated bombs containing live mice and fleas, with very small explosive loads, to deliver the weaponized microbes, overcoming the problem of the explosive killing the infected animal and insect by the use of a ceramic, rather than metal, casing for the warhead. While no records survive of the actual usage of the ceramic shells, prototypes exist and are believed to have been used in experiments during WWII.

After World War II, both the United States and the Soviet Union developed means of weaponising pneumonic plague. Experiments included various delivery methods, vacuum drying, sizing the bacterium, developing strains resistant to antibiotics, combining the bacterium with other diseases (such as diphtheria), and genetic engineering. Scientists who worked in USSR bio-weapons programs have stated that the Soviet effort was formidable and that large stocks of weaponised plague bacteria were produced. Information on many of the Soviet projects is largely unavailable. Aerosolized pneumonic plague remains the most significant threat. The plague can be easily treated with antibiotics, thus a widespread epidemic is highly unlikely in developed countries.



Worldwide distribution of plague infected animals 1998

1994 epidemic in Surat, India

In 1994, there was a pneumonic plague epidemic in Surat, India that resulted in 52 deaths and in a large internal migration of about 300,000 residents, who fled fearing quarantine.

A combination of heavy monsoon rain and clogged sewers led to massive flooding which resulted in unhygienic conditions and a number of uncleared animal carcasses. It is believed that this situation precipitated the epidemic. There was widespread fear that the flood of refugees might spread the epidemic to other parts of India and the world, but that scenario was averted, probably as a result of effective public health response mounted by the Indian health authorities.

Much like the Black Death that spread through medieval Europe, some questions still remain unanswered about the 1994 epidemic in Surat.

Initial questions about whether it was an epidemic of plague arose because the Indian health authorities were unable to culture *Yersinia pestis*, but this could have been due to poor laboratory procedures. Yet, there are several lines of evidence strongly suggesting that it was a plague epidemic: blood tests for Yersinia were positive, a number of individuals showed antibodies against Yersinia and the clinical symptoms displayed by the affected were all consistent with the disease being plague.

Other contemporary cases

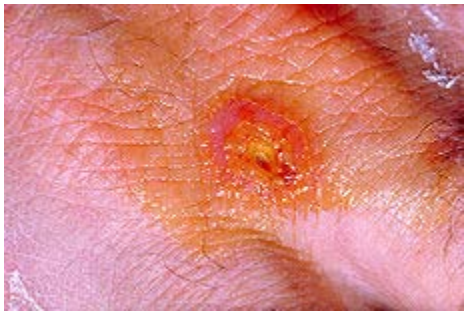
- On August 31, 1984, the Centers for Disease Control and Prevention reported a case of pneumonic plague in Claremont, California. The CDC believes that the patient, a veterinarian, contracted plague from a stray cat. As the cat wasn't available for necropsy, this could not be ultimately confirmed.
- From 1995 to 1998, annual outbreaks of plague were witnessed in Mahajanga, Madagascar.
- In the U.S., about half of all fatal cases of plague since 1970 have occurred in New Mexico. There were 2 plague deaths in the state in 2006, the first fatalities in 12 years.
- In February 2002, a small outbreak of pneumonic plague took place in the Shimla District of Himachal Pradesh state in northern India.
- In Fall of 2002, a New Mexico couple contracted the disease, just prior to a visit to New York City. They both were treated by antibiotics, but the male required amputation of both feet to fully recover, due to the lack of blood flow to his feet, cut off by the bacteria.
- On April 19, 2006, CNN News and others reported a case of plague in Los Angeles, California, lab technician Nirvana Kowlessar, the first reported case in that city since 1984.
- In May 2006, KSL Newsradio reported a case of plague found in dead field mice and chipmunks at Natural Bridges National Monument about 40 miles (64 km) west of Blanding in San Juan County, Utah.
- In May 2006, Arizona media reported a case of plague found in a cat.

- One hundred deaths resulting from pneumonic plague were reported in Ituri district of the eastern Democratic Republic of the Congo in June 2006. Control of the plague was proving difficult due to the ongoing conflict.
- It was reported in September 2006 that three mice infected with *Yersinia pestis* apparently disappeared from a laboratory belonging to the Public Health Research Institute, located on the campus of the University of Medicine and Dentistry of New Jersey, which conducts anti-bioterrorism research for the United States government.
- On May 16, 2007, an 8-year-old hooded capuchin monkey in the Denver Zoo died of the bubonic plague. Five squirrels and a rabbit were also found dead on zoo grounds and tested positive for the disease.
- On June 5, 2007 in Torrance County, New Mexico a 58 year old woman developed bubonic plague, which progressed to pneumonic plague.
- On November 2, 2007, Eric York, a 37 year old wildlife biologist for the National Park Service's Mountain Lion Conservation programPDF (144 KB) and The Felidae Conservation Fund, was found dead in his home at Grand Canyon National Park. On October 27, York performed a necropsy on a mountain lion that had likely perished from the disease and three days afterward York complained of flu-like symptoms and called in sick from work. He was treated at a local clinic but was not diagnosed with any serious ailment. The discovery of his death sparked a minor health scare, with officials stating he likely died of either plague or hantavirus, and 49 people who had come in to contact with York were given aggressive antibiotic treatments. None of them fell ill. Autopsy results released on November 9, confirmed the presence of *Y. pestis* in his body, confirming plague as a likely cause of death.
- In January 2008, at least 18 people died of bubonic plague in Madagascar.
- On June 16, 2009, Libyan authorities have reported an outbreak of bubonic plague in Tobruk, Libya. 16-18 cases were reported, including one death.
- On August 2, 2009, Chinese authorities quarantined the town of Ziketan, Xinghai County in Hainan Tibetan Autonomous Prefecture, Qinghai Province (Northwestern China) after an outbreak of pneumonic plague. As of this writing, three have died and ten more are ill, being treated in hospital.
- On September 13, 2009, dr. Malcolm Casadaban has died following an accidental laboratory exposure to an attenuated strain of the plague bacterium. This has happened due to his undiagnosed hereditary hemochromatosis (iron overload). He was an Associate Professor of Molecular Genetics and Cell Biology and of Microbiology at the University of Chicago.
- On 1 July 2010, 8 cases of Bubonic plague have been reported in humans in the District of Chicama, Peru. One 32 year old man has been affected, as well as 3 boys and 4 girls ranging in age from 8 to 14 years old. 425 houses have been fumigated and 1210 guinea pigs, 232 dogs, 128 cats and 73 rabbits have been given anti flea treatment in an effort to stop the epidemic.

Chapter 3

Tularemia

Tularemia



A tularemia lesion on the dorsal skin of right hand.

ICD-10	A21.
ICD-9	021
DiseasesDB	13454
MedlinePlus	000856
eMedicine	med/2326 emerg/591 ped/2327
MeSH	D014406

Tularemia (also known as **Pahvant Valley plague**, **rabbit fever**, **deer fly fever**, **Ohara's fever**) is a serious infectious disease caused by the bacterium *Francisella tularensis*. A Gram-negative, nonmotile coccobacillus, the bacterium has several subspecies with varying degrees of virulence. The most important of those is *F. tularensis tularensis* (Type A), which is found in lagomorphs in North America and is highly virulent in humans and domestic rabbits. *F. tularensis palaearctica* (Type B) occurs mainly in aquatic rodents (beavers, muskrats) in North America and in hares and small rodents in northern Eurasia. It is less virulent for humans and rabbits. The primary vectors are ticks and deer flies, but the disease can also be spread through other arthropods. The disease is named after Tulare County, California.

History

The bacterium was first isolated by GW McCoy of the US Public Health Service plague lab and reported in 1912. Scientists determined that tularemia could be dangerous to humans; a human being may catch the infection after contacting an infected animal. The ailment soon became frequent with hunters, cooks and agricultural workers.

Epidemiology

The disease is endemic in North America, and parts of Europe and Asia. The most common mode of transmission is via arthropod vectors. Ticks involved include *Amblyomma*, *Dermacentor*, *Haemaphysalis*, and *Ixodes*. Rodents, rabbits, and hares often serve as reservoir hosts, but waterborne infection accounts for 5 to 10% of all tularemia in the US. Tularemia can also be transmitted by biting flies, particularly the deer fly *Chrysops discalis*. Individual flies can remain infective for 14 days and ticks for over 2 years. Tularemia may also be spread by direct contact with contaminated animals or material, by ingestion of poorly cooked flesh of infected animals or contaminated water, or by inhalation. The most likely method for bioterrorist transmission is through an aerosol.

In the United States, although records show that tularemia was never particularly common, incidence rates continued to drop over the course of the 20th century so that between 1990 and 2000, the rate was less than 1 per 1,000,000, meaning the disease is extremely rare in the US today.

Clinical manifestations and microbiological diagnosis

Depending on the site of infection, tularemia has six characteristic clinical syndromes: ulceroglandular (the most common type representing 75% of all forms), glandular, oropharyngeal, pneumonic, oculoglandular, and typhoidal.

The incubation period for tularemia is 1 to 14 days; most human infections become apparent after 3 to 5 days. In most susceptible mammals, the clinical signs include fever, lethargy, anorexia, signs of septicemia, and possibly death. Animals rarely develop the skin lesions seen in people. Subclinical infections are common and animals often develop specific antibodies to the organism. Fever is moderate or very high and tularemia bacillus can be isolated from blood cultures at this stage. Face and eyes redden and become inflamed. Inflammation spreads to the lymph nodes, which enlarge and may suppurate (mimicking bubonic plague). Lymph node involvement is accompanied by a high fever. Death occurs in less than 1% if therapy is initiated promptly.

A culture of *Francisella tularensis*

The microbiologist must be informed when tularemia is suspected because *F. tularensis* requires special media for cultivation such as buffered charcoal and yeast extract (BCYE). It cannot be isolated in the routine culture media because of the need for sulfhydryl group donors (such as cystein). Serological tests (detection of antibodies in the serum of the patients) are available and widely used. Cross reactivity with *Brucella* can confuse interpretation of the results, and for this reason diagnosis should not rely only on serology. Molecular methods such as PCR are available in reference laboratories. The bacteria can penetrate into the body through damaged skin and mucous membranes, or through inhalation. Humans are most often infected by tick bite or through handling an infected animal. Ingesting infected water, soil, or food can also cause infection. Tularemia can also be acquired by inhalation; hunters are at a higher risk for this disease because of the potential of inhaling the bacteria during the skinning process. It has been contracted from inhaling particles from an infected rabbit ground up in a lawnmower. Tularemia is not spread directly from person to person.

Francisella tularensis is an intracellular bacterium, meaning that it is able to live as a parasite within host cells. It primarily infects macrophages, a type of white blood cell. It is thus able to evade the immune system. The course of disease involves spread of the organism to multiple organ systems, including the lungs, liver, spleen, and lymphatic system. The course of disease is different depending on the route of exposure. Mortality

in untreated (pre-antibiotic-era) patients has been as high as 50% in the pneumoniac and typhoidal forms of the disease, which however account for less than 10% of cases. Overall mortality was 7% for untreated cases, and the disease responds well to antibiotics with a fatality rate of about 1%. The exact cause of death is unclear, but it is thought to be a combination of multiple organ system failures.

Treatment and prevention

The drug of choice is Streptomycin. Tularemia may also be treated with gentamicin for ten days, tetracycline-class drugs such as doxycycline for 2–3 weeks, chloramphenicol or fluoroquinolones. An attenuated, live vaccine is available, but its use is only for high risk groups. Its use as post-exposure prophylaxis is not recommended.

Tularemia as a biological weapon

The Centers for Disease Control and Prevention regard *F. tularensis* as a viable bioweapons agent, and it has been included in the biological warfare programs of the USA, USSR and Japan at various times. A former Soviet biological weapons scientist, Kenneth Alibek, has alleged that an outbreak of Tularemia among German soldiers shortly before the siege of Stalingrad was due to the release of *F. tularensis* by Soviet forces, but this claim is rejected by others who have studied the outbreak. In the US, practical research into using rabbit fever as a bioweapon took place in 1954 at Pine Bluff Arsenal, Arkansas, an extension of the Camp Detrick program. It was viewed as an attractive agent because:

- it is easy to aerosolize
- it is highly infective; 10-50 bacteria are required to infect
- it is non-persistent and easy to decontaminate (unlike anthrax)
- it is highly incapacitating to infected persons
- it has comparatively low lethality, which is useful where enemy soldiers are in proximity to non-combatants, e.g. civilians

The Schu S4 strain was standardized as Agent UL for use in the U.S. M143 bursting spherical bomblet. It was a lethal biological with an anticipated fatality rate of 40 to 60 percent. The rate-of-action was around three days, with a duration-of-action of 1 to 3 weeks (treated) and 2 to 3 months (untreated) with frequent relapses. UL was streptomycin resistant. The aerobiological stability of UL was a major concern, being sensitive to sun light, and losing virulence over time after release. When the 425 strain was standardized as agent JT (an incapacitant rather than lethal agent), the Schu S4 strain's symbol was changed again to SR.

Both wet and dry types of *F. tularensis* (identified by the codes TT and ZZ) were examined during the "Red Cloud" tests, which took place from November 1966 to February 1967 in the Tanana Valley, Alaska.

No vaccine is available to the general public. The best way to prevent tularemia infection is to wear rubber gloves when handling or skinning lagomorphs (such as rabbits), avoid ingesting uncooked wild game and untreated water sources, wear long-sleeved clothes, and use an insect repellent to prevent tick bites.

Documented outbreaks

In the summer of 2000, an outbreak of tularemia in Martha's Vineyard resulted in one fatality, and brought the interest of the CDC as a potential investigative ground for aerosolized *Francisella tularensis*. Over the following summers, Martha's Vineyard was identified as the only place in the world where documented cases of tularemia resulted from lawn mowing.

An outbreak of tularemia occurred in Kosovo in 1999-2000.

In 2004, three researchers at Boston University Medical Center were accidentally infected with *F. tularensis*, after apparently failing to follow safety procedures.

In 2005, small amounts of *F. tularensis* were detected in the Mall area of Washington, DC the morning after an anti-war demonstration on September 24, 2005. Biohazard sensors were triggered at six locations surrounding the Mall. While thousands of people were potentially exposed, no infections were reported.

Tularemia is endemic in the Gori region of Eurasian country of Georgia. The last outbreak was in 2006.

In 2007, a lab of Boston University's Center for Advanced Biomedical Research, where *F. tularensis* were being kept for research, was evacuated after smoke set off alarms. An investigation has later determined that an electrical problem was the culprit, and no bacterial contamination was found.

In July 2007, an outbreak was reported in the Spanish autonomous region of Castile and León and traced to the plague of voles infesting the region. Another outbreak had taken place ten years before in the same area.

In August 2009, a Swedish tourist was bitten by an unidentified insect at Point Grey, Vancouver, BC, Canada. It was not until after return to Sweden that he was diagnosed with Tularemia, despite seeking medical treatment in Vancouver.

Chapter 4

Chagas Disease

Chagas disease

An acute Chagas disease infection with swelling of the right eye (Romaña's sign).

ICD-10	B57.
ICD-9	086
DiseasesDB	13415
MedlinePlus	001372
eMedicine	med/327
MeSH	D014355

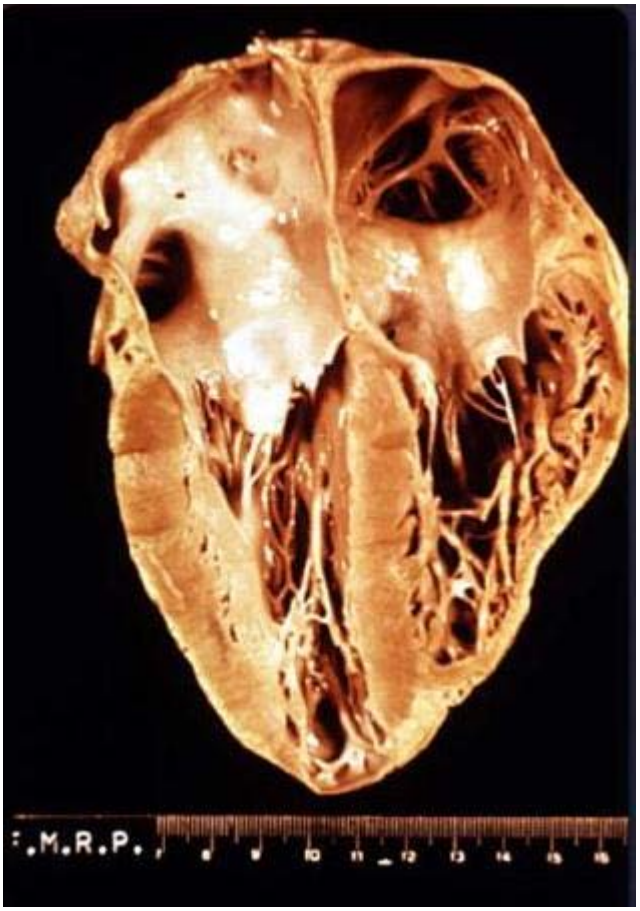
Chagas disease (Portuguese: *doença de Chagas*, Spanish: *enfermedad de Chagas-Mazza*, *mal de Chagas* in both languages; also called **American trypanosomiasis**) is a tropical

parasitic disease caused by the flagellate protozoan *Trypanosoma cruzi*. *T. cruzi* is commonly transmitted to humans and other mammals by an insect vector, the blood-sucking bugs of the subfamily Triatominae (family Reduviidae) most commonly species belonging to the *Triatoma*, *Rhodnius*, and *Panstrongylus* genera. The disease may also be spread through blood transfusion and organ transplantation, ingestion of food contaminated with parasites, and from a mother to her fetus.

The symptoms of Chagas disease vary over the course of an infection. In the early, acute stage, symptoms are mild and usually produce no more than local swelling at the site of infection. The initial acute phase is responsive to antiparasitic treatments, with 60-90% cure rates. After 4–8 weeks, individuals with active infections enter the chronic phase of Chagas disease that is asymptomatic for 60-80% of chronically infected individuals through their lifetime. The antiparasitic treatments also appear to delay or prevent the development of disease symptoms during the chronic phase of the disease, but 20-40% of chronically infected individuals will still eventually develop life-threatening heart and digestive system disorders. The currently available antiparasitic treatments for Chagas disease are benznidazole and nifurtimox, which can cause temporary side effects in many patients including skin disorders, brain toxicity, and digestive system irritation.

Chagas disease is contracted primarily in the Americas, particularly in poor, rural areas of Mexico, Central America, and South America; very rarely, the disease has originated in the Southern United States. The insects that spread the disease are known by various local names, including *vinchuca* in Argentina, Bolivia, Chile and Paraguay, *barbeiro* (the barber) in Brazil, *pito* in Colombia, *chinche* in Central America, *chipo* in Venezuela, *chupança*, *chinchorro*, and "the kissing bug". It is estimated that as many as 8 to 11 million people in Mexico, Central America, and South America have Chagas disease, most of whom do not know they are infected. Large-scale population movements from rural to urban areas of Latin America and to other regions of the world have increased the geographic distribution of Chagas disease, and cases have been noted in many countries, particularly in Europe. Control strategies have mostly focused on eliminating the triatomine insect vector and preventing transmission from other sources.

Signs and symptoms



Gross anatomy of a heart that has been damaged by chronic Chagas disease

The human disease occurs in two stages: an acute stage, which occurs shortly after an initial infection, and a chronic stage that develops over many years.

The acute phase lasts for the first few weeks or months of infection. It usually occurs unnoticed because it is symptom free or exhibits only mild symptoms that are not unique to Chagas disease. These can include fever, fatigue, body aches, headache, rash, loss of appetite, diarrhea, and vomiting. The signs on physical examination can include mild enlargement of the liver or spleen, swollen glands, and local swelling (a chagoma) where the parasite entered the body. The most recognized marker of acute Chagas disease is called Romaña's sign, which includes swelling of the eyelids on the side of the face near the bite wound or where the bug feces were deposited or accidentally rubbed into the eye. Rarely, young children, or adults may die from the acute disease due to severe inflammation/infection of the heart muscle (myocarditis) or brain (meningoencephalitis). The acute phase also can be severe in people with weakened immune systems.

If symptoms develop during the acute phase, they usually resolve spontaneously within 3–8 weeks in approximately 90% of individuals. Although the symptoms resolve, even with treatment the infection persists and enters a chronic phase. Of individuals with

chronic Chagas disease, 60-80% will never develop symptoms (called *indeterminate* chronic Chagas disease), while the remaining 20-40% will develop life-threatening heart and/or digestive disorders during their lifetime (called *determinate* chronic Chagas disease). In 10% of individuals the disease progresses directly from the acute form to a symptomatic clinical form of chronic Chagas disease.

The symptomatic (determinate) chronic stage affects the nervous system, digestive system and heart. About two thirds of people with chronic symptoms have cardiac damage, including cardiomyopathy, which causes heart rhythm abnormalities and may result in sudden death. About one third of patients go on to develop digestive system damage, resulting in dilation of the digestive tract (megacolon and megaesophagus), accompanied by severe weight loss. Swallowing difficulties (secondary achalasia) may be the first symptom of digestive disturbances and may lead to malnutrition. Twenty to fifty percent of individuals with intestinal involvement also exhibit cardiac involvement. Up to 10% of chronically infected individuals develop neuritis that results in altered tendon reflexes and sensory impairment. Isolated cases exhibit central nervous system involvement, including dementia, confusion, chronic encephalopathy and sensitivity and motor deficits.

The clinical manifestations of Chagas disease are due to cell death in the target tissues that occurs during the infective cycle, by sequentially inducing an inflammatory response, cellular lesions, and fibrosis. For example, intracellular amastigotes destroy the intramural neurons of the autonomic nervous system in the intestine and heart, leading to megaintestine and heart aneurysms, respectively. If left untreated, Chagas disease can be fatal, in most cases due to heart muscle damage.

Transmission

Rhodnius prolixus is the principal vector in Colombia, Venezuela, Guatemala, Honduras and some parts of Nicaragua and El Salvador.

In Chagas-endemic areas, the main mode of transmission is through an insect vector called a triatomine bug. A triatomine becomes infected with *T. cruzi* by feeding on the blood of an infected person or animal. During the day, triatomines hide in crevices in the walls and roofs. The bugs emerge at night, when the inhabitants are sleeping. Because they tend to feed on people's faces, triatomine bugs are also known as "kissing bugs." After they bite and ingest blood, they defecate on the person. Triatomines pass *T. cruzi* parasites (called trypomastigotes) in feces left near the site of the bite wound. Scratching the site of the bite causes the trypomastigotes to enter the host through the wound, or through intact mucous membranes, such as the conjunctiva. Once inside the host, the trypomastigotes invade cells, where they differentiate into intracellular amastigotes. The amastigotes multiply by binary fission and differentiate into trypomastigotes, which are then released into the bloodstream. This cycle is repeated in each newly infected cell.

Replication resumes only when the parasites enter another cell or are ingested by another vector.

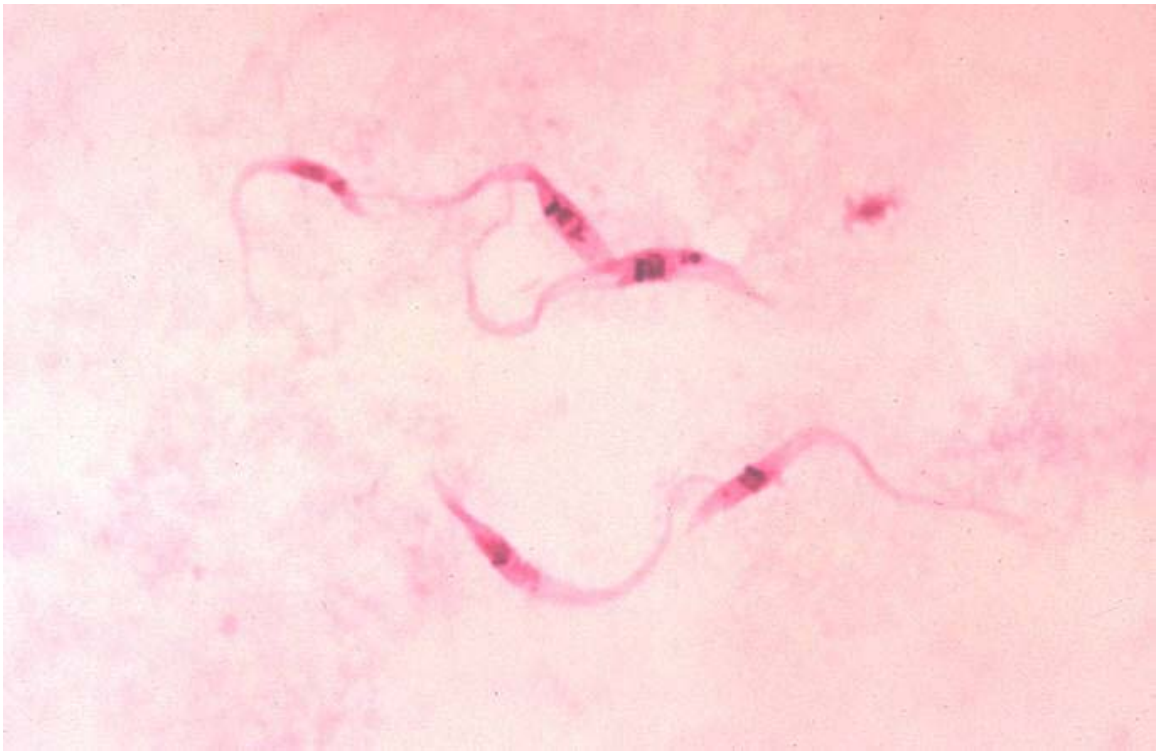
Dense vegetation (such as that of tropical rainforests) and urban habitats are not ideal for the establishment of the human transmission cycle. However, in regions where the sylvatic habitat and its fauna are thinned by economical exploitation and human habitation, such as in newly deforested areas, piassava palm culture areas, and some parts of the Amazon region, a human transmission cycle may develop as the insects search for new food sources.

T. cruzi can also be transmitted through blood transfusions. With the exception of blood derivatives (such as fractionated antibodies), all blood components are infective. The parasite remains viable at 4°C for at least 18 days or up to 250 days when kept at room temperature. It is unclear whether *T. cruzi* can be transmitted through frozen-thawed blood components.

Other modes of transmission include organ transplantation, through breast milk, and by accidental laboratory exposure. Chagas disease can also be spread congenitally (from a pregnant woman to her baby) through the placenta, and accounts for approximately 13% of stillborn deaths in parts of Brazil.

In 1991, farm workers in the state of Paraíba, Brazil, were infected by eating contaminated food; transmission has also occurred via contaminated açaí palm fruit juice and sugar cane juice. A 2007 outbreak in 103 Venezuelan school children was attributed to contaminated guava juice.

Diagnosis

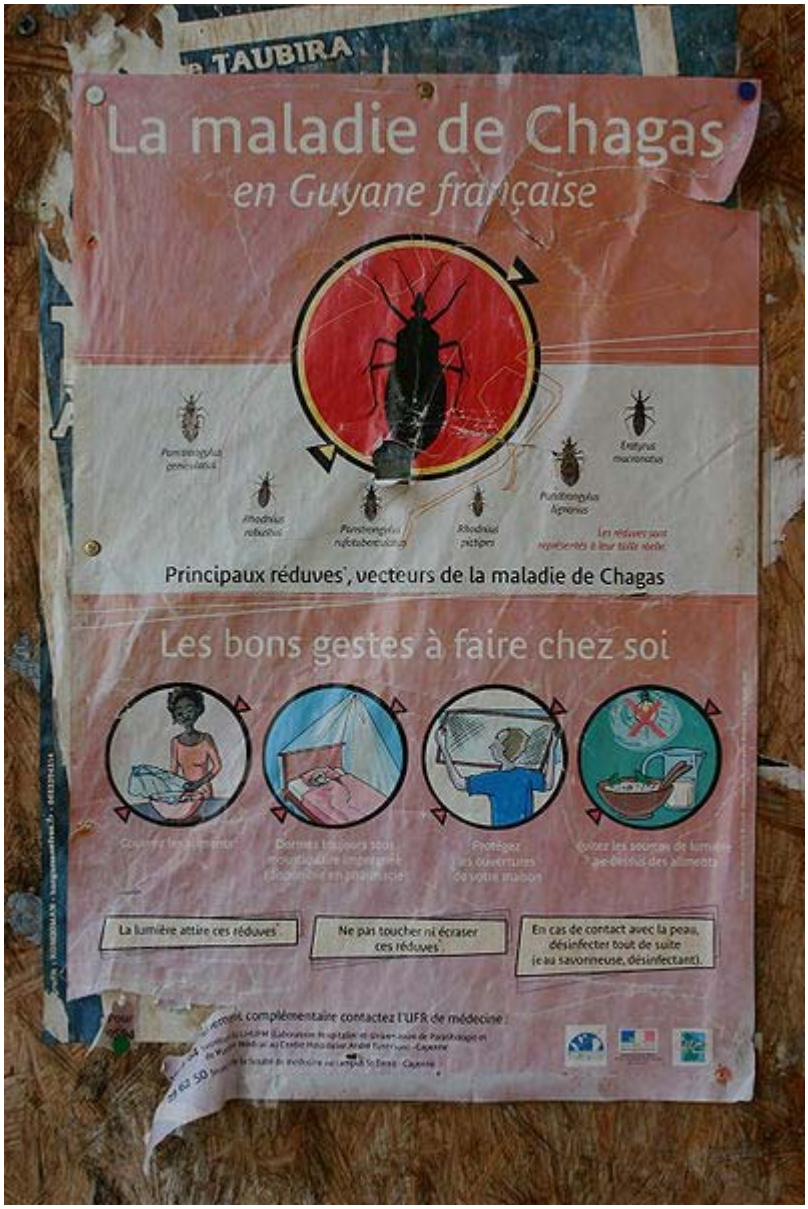


Photomicrograph of Giemsa-stained *Trypanosoma cruzi* (CDC)

The presence of *T. cruzi* is diagnostic of Chagas disease. It can be detected by microscopic examination of fresh anticoagulated blood, or its buffy coat, for motile parasites; or by preparation of thin and thick blood smears stained with Giemsa, for direct visualization of parasites. Microscopically, *T. cruzi* can be confused with *Trypanosoma rangeli*, which is not known to be pathogenic in humans. Isolation of *T. cruzi* can occur by inoculation into mice, by culture in specialized media (e.g., NNN, LIT); and by xenodiagnosis, where uninfected Reduviidae bugs are fed on the patient's blood, and their gut contents examined for parasites.

Various immunoassays for *T. cruzi* are available and can be used to distinguish among strains (zymodemes of *T.cruzi* with divergent pathogenicities). These tests include: detecting complement fixation, indirect hemagglutination, indirect fluorescence assays, radioimmunoassays, and ELISA. Alternatively, diagnosis and strain identification can be made using polymerase chain reaction (PCR).

Prevention



Awareness and prevention campaign poster in Cayenne, French Guiana, 2008

There is currently no vaccine against Chagas disease and prevention is generally focused on fighting the vector *Triatoma* by using sprays and paints containing insecticides (synthetic pyrethroids), and improving housing and sanitary conditions in rural areas. For urban dwellers, spending vacations and camping out in the wilderness or sleeping at hostels or mud houses in endemic areas can be dangerous; a mosquito net is recommended. Some stepstones of vector control include:

- A yeast trap tested for monitoring infestations of certain species of triatomine bugs (*Triatoma sordida*, *Triatoma brasiliensis*, *Triatoma pseudomaculata*, and *Panstrongylus megistus*).
- Promising results were gained with the treatment of vector habitats with the fungus *Beauveria bassiana*.
- Targeting the symbionts of Triatominae through paratransgenesis.

A number of potential vaccines are currently being tested. Vaccination with *Trypanosoma rangeli* has produced positive results in animal models. More recently, the potential of DNA vaccines for immunotherapy of acute and chronic Chagas disease is being tested by several research groups.

Blood transfusion was formerly the second most common mode of transmission for Chagas disease, but the development and implementation of blood bank screening tests has dramatically reduced this risk in the last decade. Blood donations in all endemic Latin American countries undergo Chagas screening, and testing is expanding in countries, such as France, Spain and the United States, that have significant or growing populations of immigrants from endemic areas. In Spain, donors are evaluated with a questionnaire to identify individuals at risk of Chagas exposure for screening tests. The US FDA has approved two Chagas tests including one recently approved in April 2010, and has published guidelines that recommend testing of all donated blood and tissue products. While these tests are not required in U.S., it is estimated that 75-90% of the blood supply is currently tested for Chagas, including all units collected by the American Red Cross which accounts for 40% of the U.S. blood supply. The Chagas Biovigilance Network reports current incidents of Chagas positive blood products in the United States, as reported by labs using the screening test approved by the FDA in 2007.

Management

There are two approaches to treating Chagas disease, antiparasitic treatment, to kill the parasite; and symptomatic treatment, to manage the symptoms and signs of infection.

Medication

Antiparasitic treatment is most effective early in the course of infection, but is not limited to cases in the acute phase. Drugs of choice include azole or nitro derivatives such as benznidazole or nifurtimox. Both agents are limited in their capacity to effect parasitologic cure (a complete elimination of *T. cruzi* from the body), especially in chronically infected patients, and resistance to these drugs has been reported. Studies suggest that antiparasitic treatment leads to parasitological cure in about 60-85% of adults and more than 90% of infants treated in the first year of acute phase Chagas disease. Children (age 6 to 12-years) with chronic disease have a cure rate of about 60% with benznidazole. While the rate of cure declines the longer an adult has been infected with Chagas, treatment with benznidazole has been shown to slow the onset of heart disease in adults with chronic Chagas infections.

Treatment of chronic infection in women prior to or during pregnancy does not appear to reduce the probability the disease will be passed on to the infant. Likewise, it is unclear whether prophylactic treatment of chronic infection is beneficial in persons who will undergo immunosuppression (e.g., organ transplant recipients) or in persons who are already immunosuppressed (e.g., those with HIV infection).

Complications

In the chronic stage, treatment involves managing the clinical manifestations of the disease. For example, pacemakers and medications for irregular heartbeats, such as the anti-arrhythmia drug amiodarone, may be life saving for some patients with chronic cardiac disease, while surgery may be required for megaintestine. The disease cannot be cured in this phase, however. Chronic heart disease caused by Chagas disease is now a common reason for heart transplantation surgery. Until recently, however, Chagas disease was considered a contraindication for the procedure, since the heart damage could recur as the parasite was expected to seize the opportunity provided by the immunosuppression that follows surgery. It was noted that survival rates in Chagas patients could be significantly improved by using lower dosages of the immunosuppressant drug ciclosporin. Recently, direct stem cell therapy of the heart muscle using bone marrow cell transplantation has been shown to dramatically reduce risks of heart failure in Chagas patients.

Experimental treatments

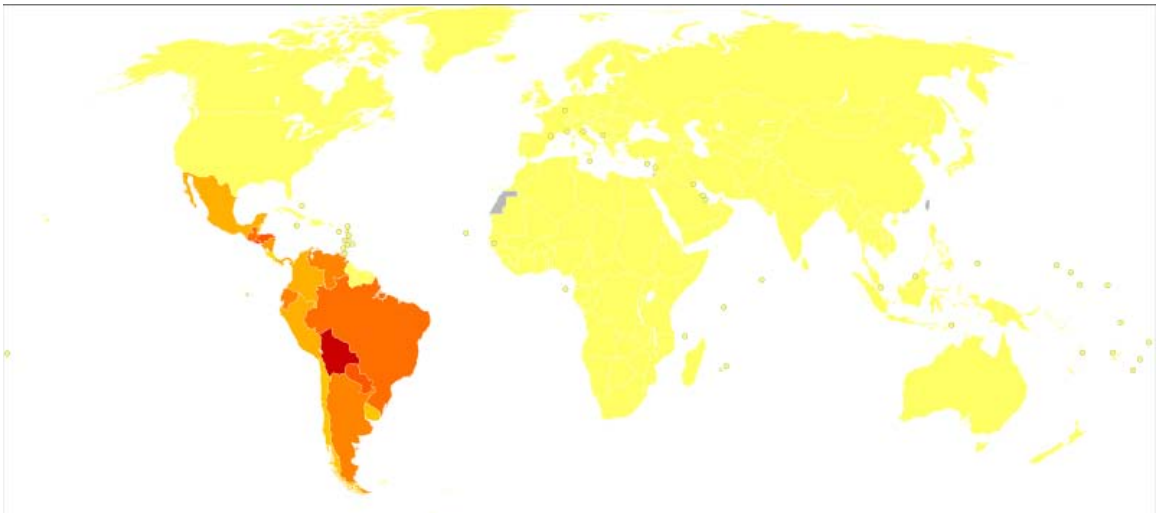
Several experimental treatments have shown promise in animal models. These include inhibitors of oxidosqualene cyclase and squalene synthase, cysteine protease inhibitors, dermaseptins collected from frogs in the genus *Phyllomedusa* (*P. oreades* and *P. distincta*), the sesquiterpene lactone dehydroleucodine (DhL) which affects the growth of cultured epimastigote–phase *Trypanosoma cruzi*, inhibitors of purine uptake, and inhibitors of enzymes involved in trypanothione metabolism. It is hoped that new drug targets may be revealed following the sequencing of the *T. cruzi* genome.

Epidemiology

Chagas in Latin America (A: Endemic zones)

Chagas disease affects 8-10 million people living in endemic Latin American countries, with and additional 300,000-400,000 living in non-endemic countries including Spain and the United States. An estimated 41,200 new cases occur annually in endemic countries and that 14,400 infants are born with congenital Chagas disease annually. About 20,000 deaths are attributed to Chagas disease each year.

The disease is present in 18 countries on the American continents, ranging from the southern United States to northern Argentina. Chagas exists in two different ecological zones. In the Southern Cone region the main vector lives in and around human homes. In Central America and Mexico the main vector species lives both inside dwellings and in uninhabited areas. In both zones Chagas occurs almost exclusively in rural areas, where triatomine breed and feed on the over 150 species from 24 families of domestic and wild mammals, as well as humans, that are the natural reservoirs of *T.cruzi*. Although Triatominae bugs feed on birds, they appear to be immune against infection and therefore are not considered to be a *T. cruzi* reservoir. Even when colonies of insects are eradicated from a house and surrounding domestic animal shelters, they can re-emerge from plants or animals that are part of the ancient, sylvatic (referring to wild animals) infection cycle. This is especially likely in zones with mixed open savannah, with clumps of trees interspersed by human habitation.



Disability-adjusted life year for chagas disease per 100,000 inhabitants in 2004.

- no data
- < 10
- 10-20
- 20-30
- 30-40
- 40-50
- 50-75
- 75-100
- 100-150
- 150-200
- 200-250
- 250-450
- > 450

The primary wildlife reservoirs for *Trypanosoma cruzi* in the United States include opossums, raccoons, armadillos, squirrels, woodrats and mice. Opossums are particularly important as reservoirs because the parasite can complete its life cycle in the anal glands of the animal without having to re-enter the insect vector. Recorded prevalence of the disease in opossums in the U.S. ranges from 8.3% up to 37.5%. Studies on raccoons in the Southeast have yielded infection rates ranging from 47% to as low as 15.5%. Armadillo prevalence studies have been described in Louisiana and range from a low of 1.1% up to 28.8%. Additionally small rodents including squirrels, mice and rats are important in the sylvatic transmission cycle because of their importance as bloodmeal sources for the insect vectors. A Texas study revealed 17.3% percent *T. cruzi* prevalence in 75 specimens representing four separate small rodent species.

Chronic Chagas disease remains a major health problem in many Latin American countries, despite the effectiveness of hygienic and preventive measures, such as eliminating the transmitting insects. However, several landmarks have been achieved in the fight against Chagas disease in Latin America including a reduction by 72% of the incidence of human infection in children and young adults in the countries of the

Southern Cone Initiative, and at least three countries (Uruguay, in 1997, and Chile, in 1999, and Brazil in 2006) have been certified free of vectorial and transfusional transmission. In Argentina vectorial transmission has been interrupted in 13 of the 19 endemic provinces. and major progress toward this goal has also been made in both Paraguay and Bolivia.

Screening of donated blood, blood components, solid organ donors, as well as donors of cells, tissues and cell and tissue products for *T. cruzi* is mandated in all Chagas endemic countries and has been implemented. Approximately 300,000 infected people live in the United States, which is likely the result of immigration from Latin American countries. With increased population movements, the possibility of transmission by blood transfusion became more substantial in the United States. Transfusion blood and tissue products are now actively screened in the U.S., thus addressing and minimizing this risk.

History

Carlos Chagas, in his laboratory at the Instituto Oswaldo Cruz

The disease was named after the Brazilian physician and infectologist Carlos Chagas, who first described it in 1909, but the disease was not seen as a major public health problem in humans until the 1960s (the outbreak of Chagas disease in Brazil in the 1920s went widely ignored). He discovered that the intestines of Triatomidae (now Reduviidae: Triatominae) harbored a flagellate protozoan, a new species of the *Trypanosoma* genus, and was able to prove experimentally that it could be transmitted to marmoset monkeys that were bitten by the infected bug. Later studies showed that squirrel monkeys were also vulnerable to infection.

Chagas named the pathogenic parasite *Trypanosoma cruzi* and later that year as *Schizotrypanum cruzi*, both honoring Oswaldo Cruz, the noted Brazilian physician and

epidemiologist who fought successfully epidemics of yellow fever, smallpox, and bubonic plague in Rio de Janeiro and other cities in the beginning of the 20th century. Chagas' work is unique in the history of medicine because he was the only researcher so far to describe *solely* and completely a new infectious disease: its pathogen, vector, host, clinical manifestations, and epidemiology.

Nevertheless, he believed (falsely) until 1925 that the main infection route was by the bite of the insect—and not by its feces, as was proposed by his colleague Emile Brumpt in 1915 and assured by Silveira Dias in 1932, Cardoso in 1938, and Brumpt himself in 1939. Chagas was also the first to unknowingly discover and illustrate the parasitic fungal genus *Pneumocystis*, later infamously to be linked to PCP (Pneumocystis pneumonia in AIDS victims). Confusion between the two pathogens' life-cycles led him to briefly recognize his genus *Schizotrypanum*, but following the description of *Pneumocystis* by others as an independent genus, Chagas returned to the use of the name *Trypanosoma cruzi*.

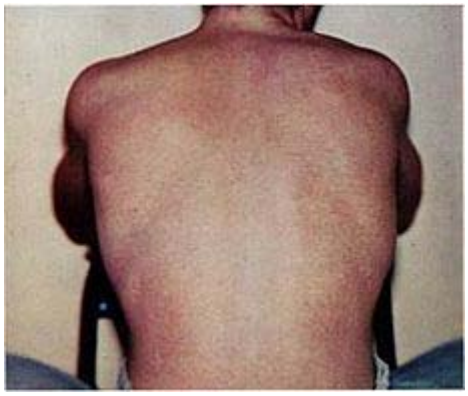
In Argentina, the disease is known as *Mal de Chagas-Mazza*, in honor of Salvador Mazza, the Argentine physician who in 1926 began investigating the disease and over the years became the principal researcher of this disease in the country. Mazza produced the first scientific confirmation of the existence of *Trypanosoma cruzi* in Argentina in 1927, eventually leading to support from local and European medical schools and Argentine government policy makers.

It has been hypothesized that Charles Darwin might have suffered from Chagas disease as a result of a bite of the so-called Great Black Bug of the Pampas (vinchuca). The episode was reported by Darwin in his diaries of the Voyage of the Beagle as occurring in March 1835 to the east of the Andes near Mendoza. Darwin was young and generally in good health, though six months previously he had been ill for a month near Valparaiso, but in 1837, almost a year after he returned to England, he began to suffer intermittently from a strange group of symptoms, becoming incapacitated for much of the rest of his life. Attempts to test Darwin's remains at the Westminster Abbey by using modern PCR techniques were met with a refusal by the Abbey's curator.

Chapter 5

Dengue Fever

Dengue fever



The typical rash seen in dengue fever

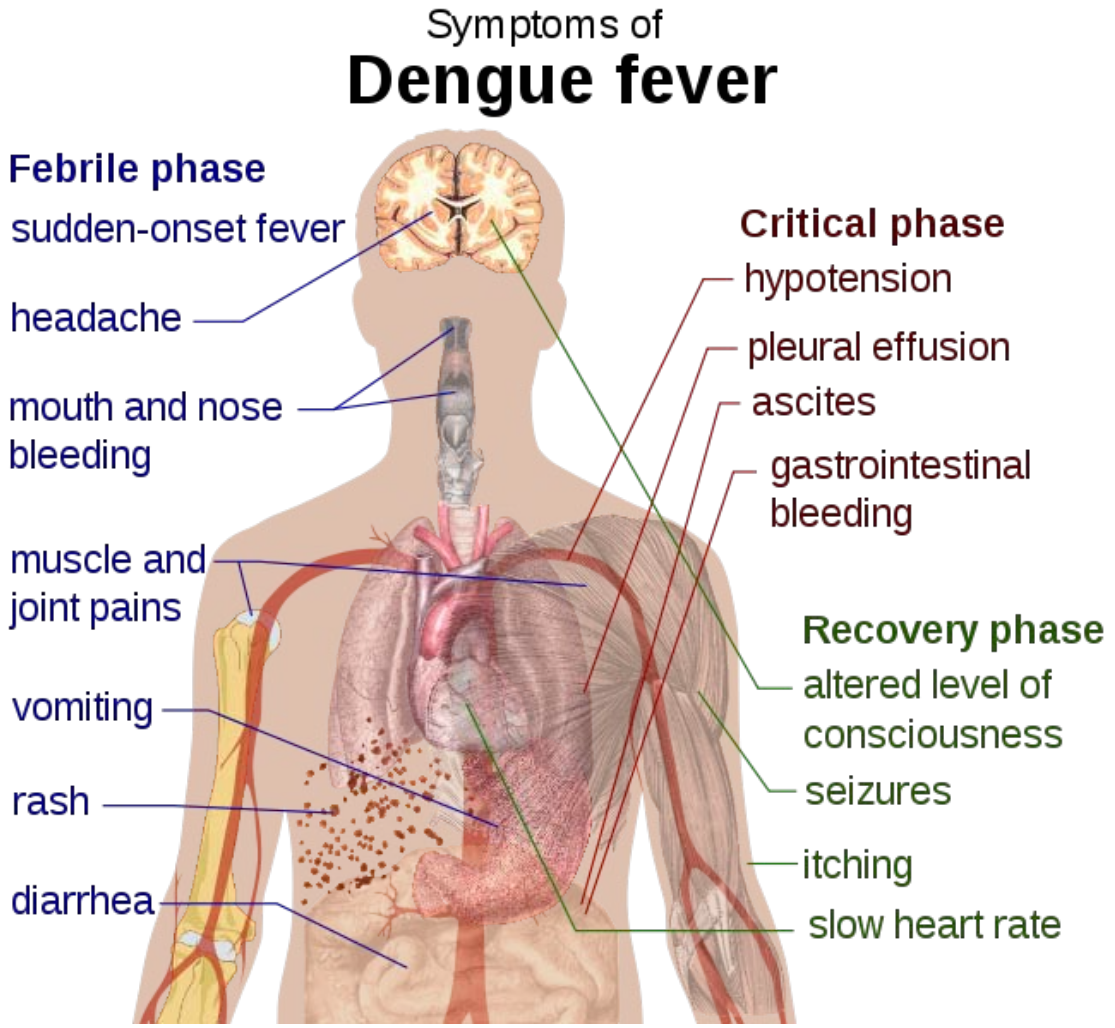
ICD-10	A90.
ICD-9	061
DiseasesDB	3564
MedlinePlus	001374
eMedicine	med/528
MeSH	*C02.782.417.214*

Dengue fever is an acute febrile infectious disease caused by the dengue virus. Typical symptoms include headache, a characteristic skin rash, and muscle and joint pains; in a small proportion the disease progresses to life-threatening complications such as **dengue hemorrhagic fever** (which may lead to severe hemorrhage) and **dengue shock syndrome** (where a very low blood pressure can cause organ dysfunction).

Dengue is usually transmitted by the mosquito *Aedes aegypti*, and rarely *Aedes albopictus*. The virus exists in four different types, and an infection with one type usually gives lifelong immunity to that type, but only short-term immunity to the others. There is currently no available vaccine, but measures to reduce the habitat and the number of mosquitoes, and limiting exposure to bites, are used to decrease the incidence of dengue.

Treatment of acute dengue is supportive, using either oral or intravenous rehydration for mild or moderate disease, and intravenous fluids and blood transfusions for more severe cases. The rate of infection has increased dramatically over the last 50 years, with around 50–100 million people being infected yearly. A global disease, dengue is currently endemic in more than 110 countries. Early descriptions of the condition date from 1779, and its viral cause and the transmission were elucidated in the early 20th century. Dengue has become a worldwide problem since the Second World War.

Signs and symptoms



Schematic depiction of the symptoms of dengue fever

People infected with dengue virus are commonly asymptomatic or only have mild symptoms such as an uncomplicated fever. Others have much more severe illness, and in a small proportion it is life-threatening. The incubation period (time between exposure and onset of symptoms) ranges from 3–14 days, but most often it is 4–7 days. This means that travellers returning from endemic areas are unlikely to have dengue if fever or other symptoms start more than 14 days after arriving home. Children often experience

symptoms similar to those of the common cold and gastroenteritis (vomiting and diarrhea), but are more susceptible to the severe complications.

Clinical course

The characteristic symptoms of dengue are: a sudden-onset fever, headache (typically behind the eyes), muscle and joint pains, and a rash; the alternative name for dengue, "break-bone fever", comes from the associated muscle and joints pains. The course of infection is divided into three phases: febrile, critical, and recovery.

The febrile phase involves high fevers, frequently over 40 °C (104 °F) and associated with generalized pain and a headache; this usually lasts 2–7 days. Flushed skin and some small red spots called petechia, which are caused by broken capillaries, may occur at this point, as may some mild bleeding from mucous membranes of the mouth and nose.

The critical phase, if it occurs, follows the resolution of the high fever and typically lasts one to two days. During this phase there may be significant fluid accumulation in the chest and abdominal cavity due to increased capillary permeability and leakage. This leads to depletion of fluid from the circulation and decreased blood supply to vital organs. During this phase, organ dysfunction and severe bleeding (typically from the gastrointestinal tract) may occur. Shock and hemorrhage occur in less than 5% of all cases of dengue but those who have previously been infected with other serotypes of dengue virus ("secondary infection") have an increased risk of this.

The recovery phase occurs next, with resorption of the leaked fluid into the bloodstream. This usually occurs over a period of two to three days. The improvement is often striking, but there may be severe itching and a slow heart rate. It is during this stage that a fluid overload state may occur, which if it affects the brain may reduce the level of consciousness or cause seizures.

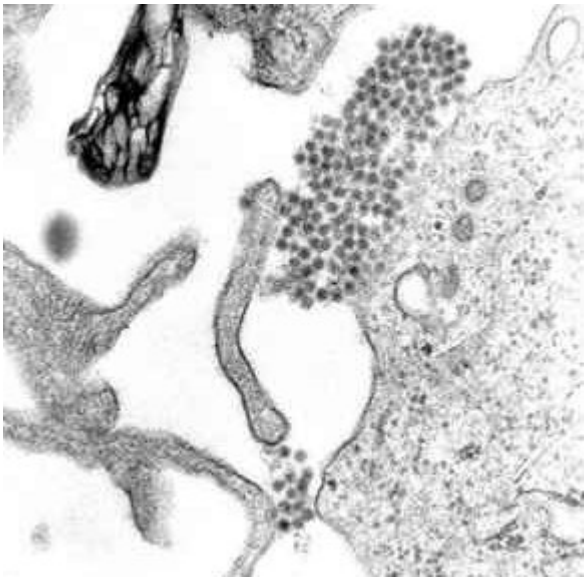
Associated problems

Dengue may occasionally affect several other body systems. This may be either in isolation or along with the classic dengue symptoms. A decreased level of consciousness occurs in 0.5–6% of severe cases. This may be caused by infection of the brain by the virus or indirectly due to impairment of vital organs, for example, the liver.

Other neurological disorders has been reported in the context of dengue, such as transverse myelitis and Guillain-Barré syndrome. Infection of the heart and acute liver failure are among the rarer complications of dengue.

Cause

Virology



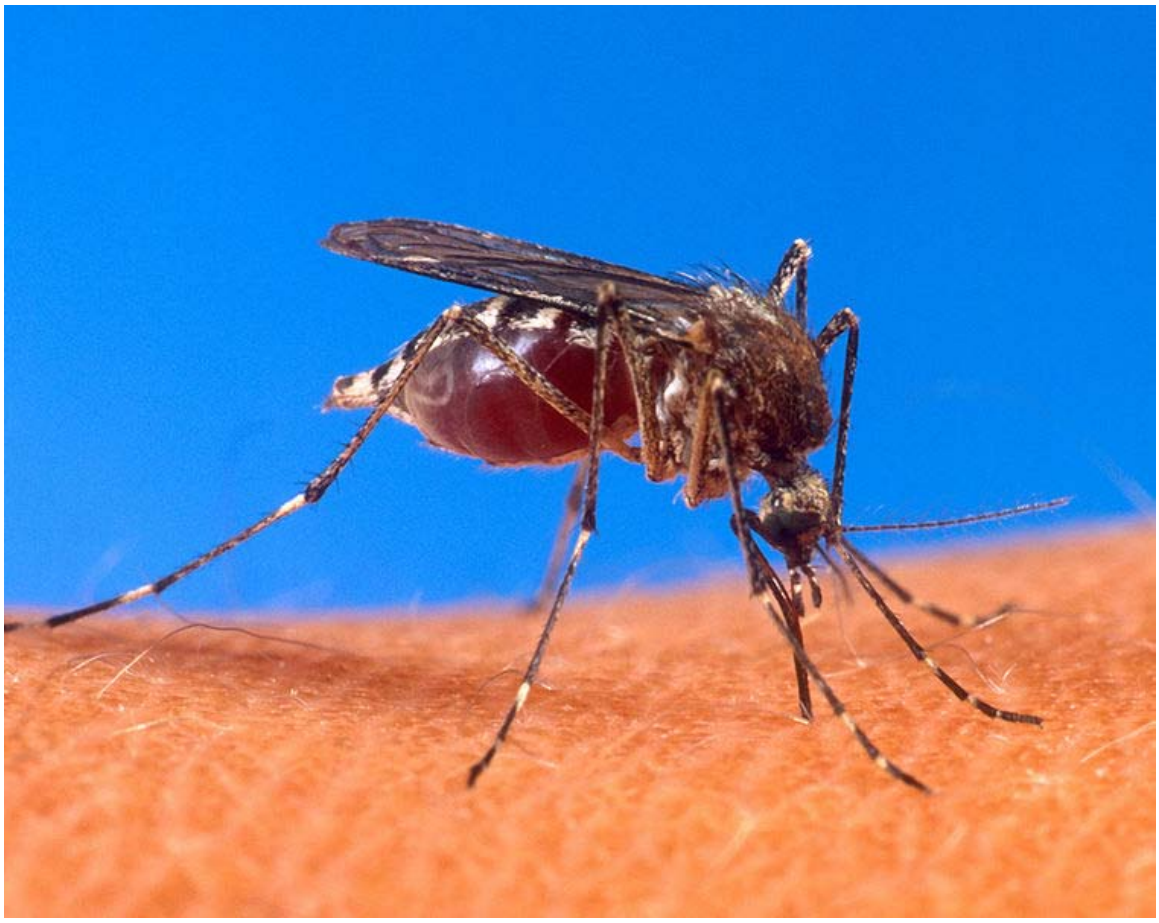
A TEM micrograph showing dengue virus virions (the cluster of dark dots near the center)

Dengue fever virus (DENV) is an RNA virus of the family *Flaviviridae*; genus *Flavivirus*. Other members of the same family include yellow fever virus, West Nile virus, St. Louis encephalitis virus, Japanese encephalitis virus, tick-borne encephalitis virus, Kyasanur forest disease virus, and Omsk hemorrhagic fever virus. Most are transmitted by arthropods (mosquitoes or ticks), and are therefore also referred to as arboviruses (arthropod-borne viruses).

The dengue virus genome (genetic material) contains of about 11,000 nucleotide bases, which code for the three different types of protein molecules that form the virus particle (C, prM and E) and seven other types of protein molecules (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) that are only found in infected host cells and are required for replication of the virus. There are four strains of the virus, which are called serotypes, and these are referred to as DENV-1, DENV-2, DENV-3 and DENV-4. All four serotypes can cause the full spectrum of disease. Infection with one serotype is believed to produce lifelong immunity to it but only short term protection against the others.

The severe complications on secondary infection seem to occur particularly if someone previously exposed to serotype DENV-1 then contracts serotype DENV-2 or serotype DENV-3, or if someone previously exposed to type DENV-3 then acquires DENV-2.

Transmission



The mosquito *Aedes aegypti* feeding off a human host

Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti*. These mosquitoes usually live between the latitudes of 35 degrees North and 35 degrees South below an elevation of 1,000 metres (3,300 ft). They bite primarily during the day. Other mosquito species—*A. albopictus*, *A polynesiensis* and several *A. scutellaris*—may also transmit the disease. Humans are the primary host of the virus, but it may also circulate in nonhuman primates. An infection may be acquired via a single bite. A mosquito that takes a blood meal from a person infected with dengue fever becomes itself infected with the virus in the cells lining its gut. About 8–10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life. *Aedes aegypti* prefers to lay its eggs in artificial water containers and tends to live in close proximity to humans, and has a preference for feeding off them rather than other vertebrates.

Dengue may also be transmitted via infected blood products and through organ donation. In countries such as Singapore, where dengue is endemic, the risk is estimated to be between 1.6 and 6 per 10,000 transfusions. Vertical transmission (from mother to child)

during pregnancy or at birth has been observed. Other person-to-person modes of transmission have been reported, but are very unusual.

Predisposition

Severe disease is more common in babies and young children, and in contrast to many other infections it is more common in children that are relatively well nourished. Women are more at risk than men. Dengue may be life-threatening in people with chronic diseases such as diabetes and asthma.

It is thought that polymorphisms (normal variations) in particular genes may increase the risk of severe dengue complications. Examples include the genes coding for the proteins known as TNFα, mannan-binding lectin, CTLA4, TGFβ, DC-SIGN, and particular alleles of human leukocyte antigen. A genetic abnormality common in Africans, known as glucose-6-phosphate dehydrogenase deficiency, appears to increase the risk. Polymorphisms in the genes for the vitamin D receptor and FcγR seem to offer protection.

Mechanism

When a mosquito carrying DENV bites a person, the virus enters the skin together with the mosquito's saliva. It binds to and enters white blood cells, and reproduces inside the cells while they move throughout the body. The white blood cells respond by producing a number of signalling proteins (such as interferon) that are responsble for many of the symptoms, such as the fever, the flu-like symptoms and the severe pains. In severe infection, the virus production inside the body is much increased, and many more organs (such as the liver and the bone marrow) can be affected, and fluid from the bloodstream leaks through the wall of small blood vessels into body cavities. As a result, less blood circulates in the blood vessels, and the blood pressure becomes so low that it cannot supply sufficient blood to vital organs. Furthermore, dysfunction of the bone marrow leads to reduced numbers of platelets, which are necessary for effective blood clotting; this increases the risk of bleeding, the other major complication of dengue.

Viral reproduction

After entering the skin, DENV binds to Langerhans cells (a population of dendritic cells in the skin that identifies pathogens). The virus enters the cells through binding between viral proteins and membrane proteins on the Langerhans cell, specifically the C-type lectins called DC-SIGN, mannose receptor and CLEC5A. DC-SIGN, a non-specific receptor for foreign material on dendritic cells, seems to be the main one. The dendritic cell moves to the nearest lymph node. Meanwhile, the virus genome is replicated in membrane-bound vesicles on the cell's endoplasmic reticulum, where the cell's protein synthesis apparatus produces new viral proteins, and the viral RNA is copied. Immature virus particles are transported to the Golgi apparatus, the part of the cell where the some of the proteins receive necessarily sugar chains (glycoproteins). The now mature new

viruses bud on the surface of the infected cell and are released by exocytosis. They are then able enter other white blood cells (such as monocytes and macrophages).

The initial reaction of infected cells is to produce the interferon, a cytokine that raises a number of defenses against viral infection through the innate immune system by augmenting the production of a large group of proteins (mediated by the JAK-STAT pathway). Some serotypes of DENV appear to have mechanisms to slow down this process. Interferon also activates the adaptive immune system, which leads to the generation of antibodies against the virus as well as T cells that directly attack any cell infected with the virus. Various antibodies are generated; some bind closely to the viral proteins and target them for phagocytosis (ingestion by specialized cells) and destruction, but some bind the virus less well and appear instead to deliver the virus into a part of the phagocytes where it is not destroyed but is able to replicate further.

Severe disease

It is not entirely clear why secondary infection with a different strain of DENV places people at risk of dengue hemorrhagic fever and dengue shock syndrome. The most widely accepted hypothesis is that of antibody-dependent enhancement (ADE). The exact mechanism behind ADE is unclear. It may be caused by poor binding of non-neutralizing antibodies and delivery into the wrong compartment of white blood cells that have ingested the virus for destruction. There is a suspicion that ADE is not the only mechanism underlying severe dengue-related complications, and various lines of research have implied a role for T cells and soluble factors (such as cytokines and the complement system).

Severe disease is marked by two problems: dysfunction of endothelium (the cells that line blood vessels) and disordered coagulation (blood clotting). Endothelial dysfunction leads to the leakage of fluid from the blood vessels into the chest and abdominal cavities, while coagulation disorder is responsible for the bleeding complications. Higher levels of virus in the blood and involvement of other organs (such as the bone marrow and the liver) are associated with more severe disease. Cells in the affected organs die, leading to the release of cytokines and activation of both coagulation and fibrinolysis (the opposing systems of blood clotting and clot degradation). These alterations together lead to both endothelial dysfunction and coagulation disorder.

Diagnosis

General

Warning signs
Abdominal pain
Ongoing vomiting
Liver enlargement
Mucosal bleeding
High hematocrit with low platelets

Lethargic

The diagnosis of dengue is typically made clinically, on the basis of reported symptoms and physical examination; this applies especially in endemic areas. Early disease can however be difficult to differentiate from other viral infections. A probable diagnosis is based on the findings of fever plus two of the following: nausea and vomiting, rash, generalized pains, low white blood cell count, positive tourniquet test, or any warning sign (see table) in someone who lives in an endemic area. Warning signs typically occur before the onset of severe dengue. The tourniquet test, which is particularly useful in settings where no laboratory investigations are readily available, involves the application of a blood pressure cuff for five minutes, followed by the counting of any petechial hemorrhages; a higher number makes a diagnosis of dengue more likely. It may be difficult to distinguish dengue fever and chikungunya, a similar viral infection that shares many symptoms and occurs in similar parts of the world to dengue. Often, investigations are performed to exclude other conditions that cause similar symptoms, such as malaria, leptospirosis, typhoid fever, and meningococcal disease.

The earliest change detectable on laboratory investigations is a low white blood cell count, which may then be followed by low platelets and metabolic acidosis. Plasma leakage may result in hemoconcentration (as indicated by a rising hematocrit) and hypoalbuminemia. Pleural effusions or ascites may be detected on clinical examination when large, but the demonstration of fluid on ultrasound may assist in the early identification of dengue shock syndrome. The use of ultrasound is limited by lack of availability in many settings.

Classification

The World Health Organization's 2009 classification divides dengue fever into two groups: uncomplicated and severe. This replaces the 1997 WHO classification, which was simplified as it was found to be too restrictive, but the older classification is still widely used. The 1997 classification divided dengue into undifferentiated fever, dengue fever, and dengue hemorrhagic fever. Dengue hemorrhagic fever was subdivided further into four grades (grade I–IV). Grade I is the presence only of easy bruising or a positive "tourniquet test" in someone with fever, grade II is the presence of spontaneous bleeding into the skin and elsewhere, grade III is the clinical evidence of shock, and grade IV is shock so severe that blood pressure and pulse cannot be detected. Grades III and IV are referred to as "dengue shock syndrome".

Virology and serology

Dengue fever may also be diagnosed by microbiological laboratory testing. This can be done by virus isolation in cell cultures, nucleic acid detection by PCR, viral antigen detection or specific antibodies (serology). Virus isolation and nucleic acid detection are more accurate than antigen detection, but these tests are not widely available due to their greater cost. All tests may be negative in the early stages of the disease.

Apart from serology, laboratory tests are only of diagnostic value during the acute phase of the illness. Tests for dengue virus-specific antibodies, types IgG and IgM, can be useful in confirming a diagnosis in the later stages of the infection. Both IgG and IgM are produced after 5–7 days. The highest levels (titres) of IgM are detected following a primary infection, but IgM is also produced in secondary and tertiary infections. The IgM becomes undetectable 30–90 days after a primary infection, but earlier following re-infections. IgG, by contrast, remains detectable for over 60 years and, in the absence of symptoms, is a useful indicator of past infection. After a primary infection the IgG reaches peak levels in the blood after 14–21 days. In subsequent re-infections levels peak earlier and the titres are usually higher. Both IgG and IgM provide protective immunity to the infecting serotype of the virus. In the laboratory test the IgG and the IgM antibodies can cross-react with other flaviviruses, such as yellow fever virus, which can make the interpretation of the serology difficult. The detection of IgG alone is not considered diagnostic unless blood samples are collected 14 days apart and a greater than fourfold increase in levels of specific IgG is detected. In a person with symptoms, the detection of IgM is considered diagnostic.

Prevention

A 1920s photograph of efforts to disperse standing water and thus decrease mosquito populations

There are currently no approved vaccines for the dengue virus. Prevention thus depends on control of and protection from the bites of the mosquito that transmits it. The World Health Organization recommends a Integrated Vector Control program consisting of five elements: (1) Advocacy, social mobilization and legislation to ensure that public health bodies and communities are strengthened, (2) collaboration between the health and other sectors (public and private), (3) an integrated approach to disease control to maximize use of resources, (4) evidence-based decision making to ensure any interventions are targeted appropriately and (5) capacity-building to ensure an adequate response to the local situation.

The primary method of controlling *A. aegypti* is by eliminating its habitats. This may be done by emptying containers of water or by adding insecticides or biological control agents to these areas. Reducing open collections of water through environmental modification is the preferred method of control, given the concerns of negative health effect from insecticides and greater logistical difficulties with control agents. People may prevent mosquito bites by wearing clothing that fully covers the skin and/or the application of insect repellent (DEET being the most effective).

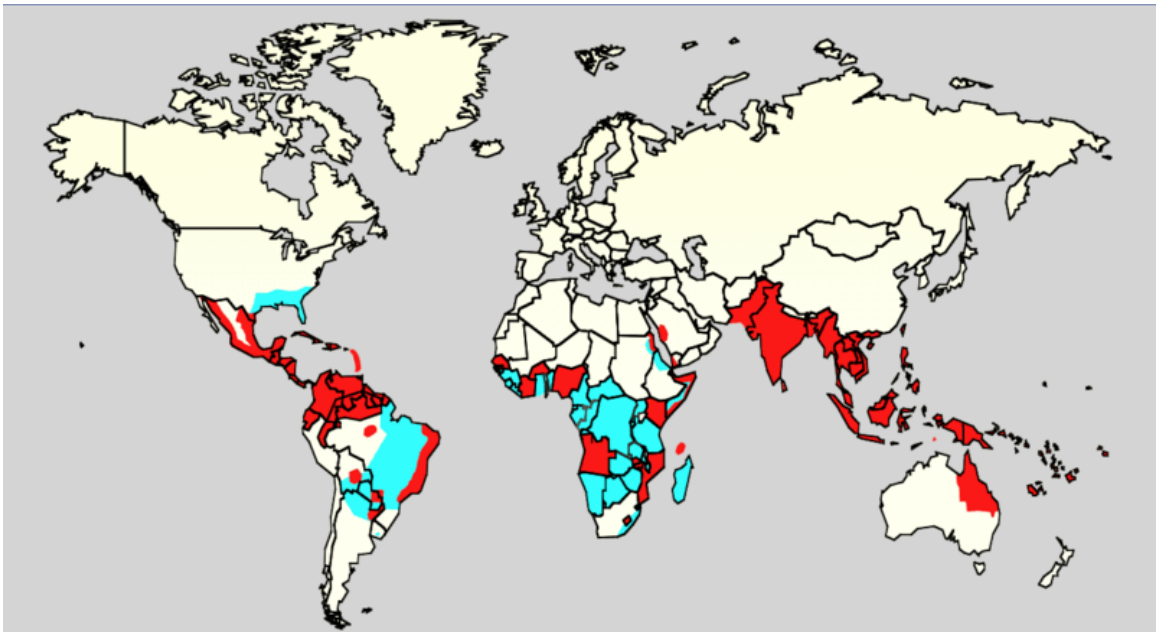
Management

There are no specific treatments for the dengue fever virus. Treatment depends on the symptoms, and may vary from advice to drink plenty of fluids such as oral rehydration solution at home with close follow up, to admission to hospital for carefully titrated isotonic intravenous fluids and/or blood transfusions. A decision for hospital admission is typically based on the presence or absence of the "warning signs" listed above, and the presence of preexisting health conditions.

Intravenous fluids if used are usually only needed for one or two days. Fluids are titrated to a urinary output of 0.5–1 mL/kg/hr, stable vital signs and normalization of hematocrit. Procedures that increase bleeding risk such as nasogastric tubes, intramuscular injections and arterial punctures are avoided. Acetaminophen may be used for fever and discomfort while NSAIDs such as ibuprofen or aspirin are avoided due to an increased bleeding risk. The need for blood transfusions is based on the presence of unstable vital signs and a decreasing haematocrit rather than the usual haematocrit of less than 30% used in sepsis. Packed red blood cells or whole blood are recommended, while platelets and fresh frozen plasma are usually not.

During the recovery phase intravenous fluids are discontinued to prevent a state of fluid overload. If fluid overload occurs and vital signs are stable, stopping further fluid may be all that is needed. If a person is outside of the critical phase, the loop diuretic furosemide may be used to eliminate excess fluid from the circulation.

Epidemiology



Dengue distribution in 2006.
Red: Epidemic dengue and *Ae. aegypti*
Aqua: Just *Ae. aegypti*.

Most people with dengue recover without any ongoing problems. The mortality is 1–5% without treatment, and less than 1% with adequate treatment. Severe disease carries a mortality of 26%. Dengue is believed to infect 50 to 100 million people worldwide a year with half a million life-threatening infections requiring hospitalization, resulting in approximately 12,500–25,000 deaths.

The burden of disease from dengue is estimated to be similar to other childhood and tropical diseases, such as tuberculosis, at 1600 disability-adjusted life years per million population. It is the most common viral disease transmitted by arthropods. As a tropical disease it is deemed only second in importance to malaria. While once exclusively a tropical disease it has become global, and is endemic in more than 110 countries. The World Health Organization counts dengue as one of sixteen neglected tropical diseases.

The incidence of dengue increased 30 fold between 1960 and 2010. This increase is believed to be due to a combination of urbanization, population growth, increased international travel, and global warming. The geographical distribution is around the equator with 70% of the total 2.5 billion people living in endemic areas from Asia and the Pacific. In the United States, the rate of dengue infection among those who return from an endemic area with a fever is 2.9–8.0%, and it is the second most common infection after malaria to be diagnosed in this group.

Until 2003, dengue was classified as a potential bioterrorism agent, but subsequent reports removed this classification as it was deemed too difficult to transfer and only caused hemorrhagic fever in a relatively small proportion of people.

History

Etymology

The origins of the word "dengue" are not clear, but one theory is that it is derived from the Swahili phrase *Ka-dinga pepo*, which describes the disease as being caused by an evil spirit. The Swahili word *dinga* may possibly have its origin in the Spanish word *dengue* meaning fastidious or careful, which would describe the gait of a person suffering the bone pain of dengue fever. Alternatively, the use of the Spanish word may derive from the similar-sounding Swahili. Slaves in the West Indies who contracted dengue were said to have the posture and gait of a dandy, and the disease was known as "dandy fever".

The term "break-bone fever" was first applied by physician and Founding Father Benjamin Rush, in a 1789 report of the 1780 epidemic in Philadelphia. In the report he uses primarily the more formal term "bilious remitting fever". The term dengue fever only came into general use after 1828. Other historical terms include "breakheart fever" and "la dengue". Terms for severe disease include: "infectious thrombocytopenic purpura" and "Philippine", "Thai", or "Singapore hemorrhagic fever".

Discovery

The first record of a case of probable dengue fever is in a Chinese medical encyclopedia from the Jin Dynasty (265–420 AD) which referred to a "water poison" associated with flying insects. There have been descriptions of epidemics in the 17th century, but the most plausible early reports of dengue epidemics are from 1779 and 1780, when an epidemic swept Asia, Africa and North America. From that time until 1940, epidemics were infrequent.

In 1906, transmission by the *Aedes* mosquitoes was confirmed, and in 1907 dengue was the second disease (after yellow fever) that was shown to be caused by a virus. Further investigations by John Burton Cleland and Joseph Franklin Siler completed the basic understanding of dengue transmission.

The marked rise of spread of dengue during and after the Second World War has been attributed to ecologic disruption. The same trends also led to the spread of different serotypes of the disease to different areas, and the emergence of dengue hemorrhagic fever, which was first reported in the Philippines in 1953. In the 1970s, it became a major cause of child mortality. Around the same time it emerged in the Pacific and the Americas. Dengue hemorrhagic fever and dengue shock syndrome were first noted in Middle and Southern America in 1981, as DENV-2 was contracted by people who had previously been infected with DENV-1 several years earlier.

Research directions

Current research efforts to prevent and treat dengue have included different means of vector control, vaccine development, and antiviral drugs.

With regards to vector control, a number of novel methods have been used to reduce mosquito numbers with some success including the placement of the fish *Poecilia reticulata* or copepods in standing water to eat the mosquito larva.

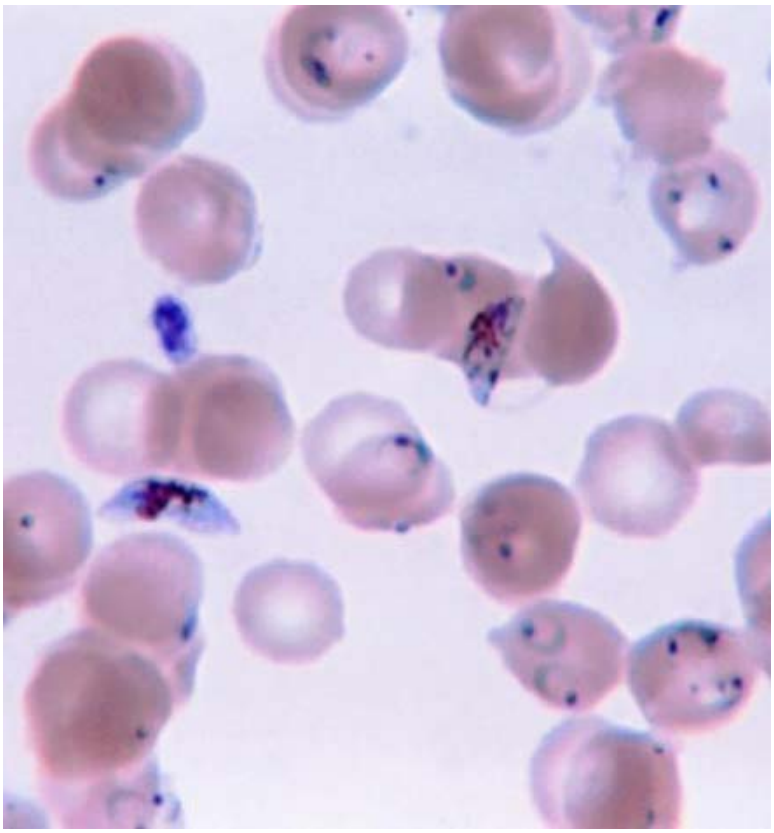
There are ongoing programs working on a dengue vaccine to cover all four serotypes. One of the concerns is that a vaccine may increase the risk of severe disease through antibody-dependent enhancement. The ideal vaccine is safe, effective after one or two injections, covers all serotypes, does not contribute to ADE, is easily transported and stored, and is both affordable and cost-effective. A number of vaccines are currently undergoing testing. It is hoped that the first products will be commercially available by 2015.

Apart from attempts to control the spread of the *Aedes* mosquito and work to develop a vaccine against dengue, there are ongoing efforts to develop antiviral drugs that might be used to treat attacks of dengue fever and prevent severe complications. Discovery of the structure of the viral proteins may aid the development of effective drugs. There are several plausible targets. The first approach is inhibition of the viral RNA-dependent RNA polymerase (coded by NS5), which copies the viral genetic material, with nucleoside analogs. Secondly, it may be possible to develop specific inhibitors of the viral protease (coded by NS3), which splices viral proteins. Finally, it may be possible to develop entry inhibitors, which stop the virus entering cells, or inhibitors of the 5' capping process, which is required for viral replication.

Chapter 6

Malaria

Malaria



Plasmodium falciparum ring-forms and gametocytes in human blood.

ICD-10	B50.
ICD-9	084
OMIM	248310
DiseasesDB	7728

MedlinePlus 000621

eMedicine med/1385 emerg/305 ped/1357

MeSH *C03.752.250.552*

Malaria is a mosquito-borne infectious disease caused by a eukaryotic protist of the genus *Plasmodium*. It is widespread in tropical and subtropical regions, including parts of the Americas (22 countries), Asia, and Africa. Each year, there are more than 250 million cases of malaria, killing between one and three million people, the majority of whom are young children in sub-Saharan Africa. Ninety percent of malaria-related deaths occur in sub-Saharan Africa. Malaria is commonly associated with poverty, and can indeed be a cause of poverty and a major hindrance to economic development.

Five species of the plasmodium parasite can infect humans: the most serious forms of the disease are caused by *Plasmodium falciparum*. Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* causes milder disease in humans that is not generally fatal. A fifth species, *Plasmodium knowlesi*, is a zoonosis that causes malaria in macaques but can also infect humans.

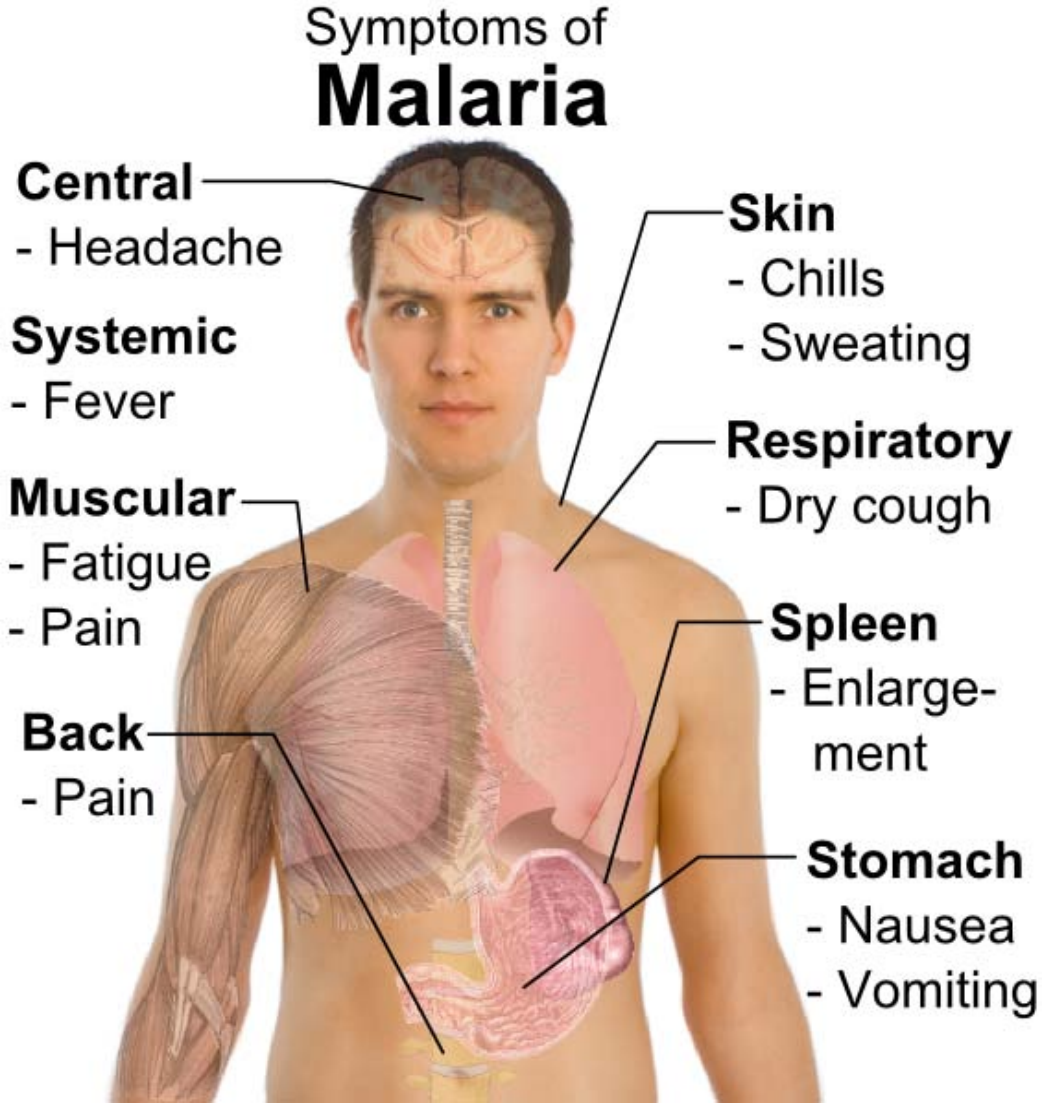
Malaria is naturally transmitted by the bite of a female *Anopheles* mosquito. When a mosquito bites an infected person, a small amount of blood is taken, which contains malaria parasites. These develop within the mosquito, and about one week later, when the mosquito takes its next blood meal, the parasites are injected with the mosquito's saliva into the person being bitten. After a period of between two weeks and several months (occasionally years) spent in the liver, the malaria parasites start to multiply within red blood cells, causing symptoms that include fever, and headache. In severe cases the disease worsens leading to hallucinations, coma, and death.

A wide variety of antimalarial drugs are available to treat malaria. In the last 5 years, treatment of *P. falciparum* infections in endemic countries has been transformed by the use of combinations of drugs containing an artemisinin derivative. Severe malaria is treated with intravenous or intramuscular quinine or, increasingly, the artemisinin derivative artesunate. Several drugs are also available to prevent malaria in travellers to malaria-endemic countries (prophylaxis). Resistance has developed to several antimalarial drugs, most notably chloroquine.

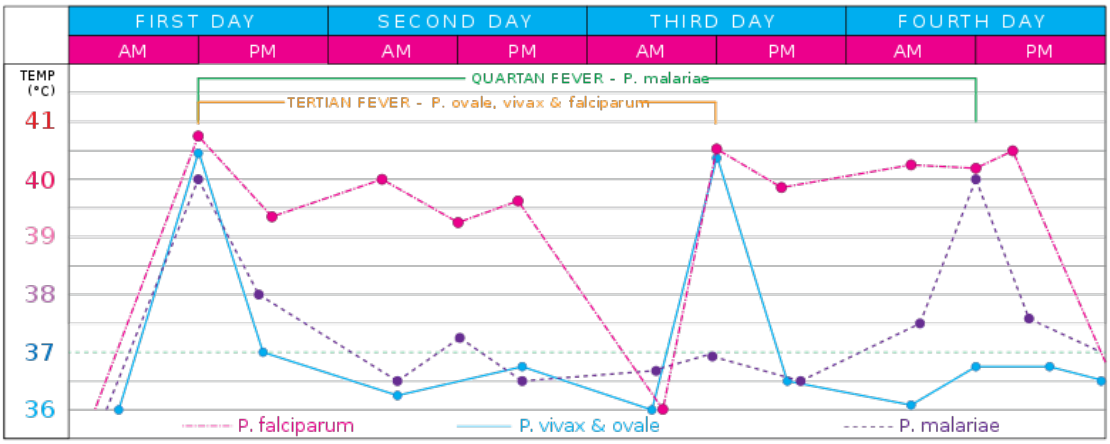
Malaria transmission can be reduced by preventing mosquito bites by distribution of inexpensive mosquito nets and insect repellents, or by mosquito-control measures such as spraying insecticides inside houses and draining standing water where mosquitoes lay their eggs.

Although many are under development, the challenge of producing a widely available vaccine that provides a high level of protection for a sustained period is still to be met.

Signs and symptoms

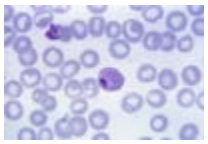
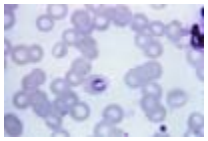
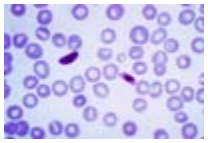
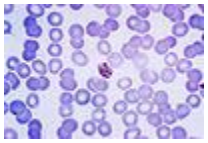


Main symptoms of malaria



Typical fever patterns in Malaria

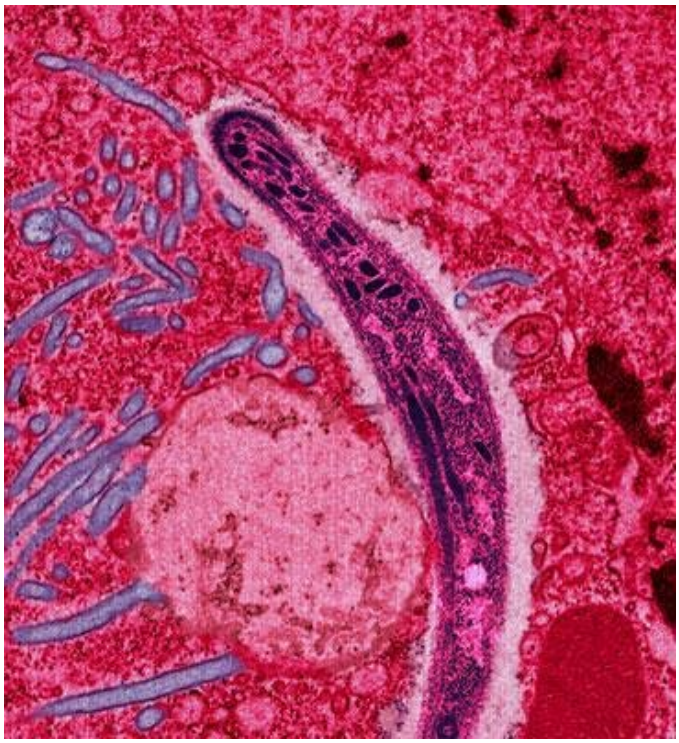
Symptoms of malaria include fever, shivering, arthralgia (joint pain), vomiting, anemia (caused by hemolysis), hemoglobinuria, retinal damage, and convulsions. The classic symptom of malaria is cyclical occurrence of sudden coldness followed by rigor and then fever and sweating lasting four to six hours, occurring every two days in *P. vivax* and *P. ovale* infections, while every three days for *P. malariae*. *P. falciparum* can have recurrent fever every 36–48 hours or a less pronounced and almost continuous fever. For reasons that are poorly understood, but that may be related to high intracranial pressure, children with malaria frequently exhibit abnormal posturing, a sign indicating severe brain damage. Malaria has been found to cause cognitive impairments, especially in children. It causes widespread anemia during a period of rapid brain development and also direct brain damage. This neurologic damage results from cerebral malaria to which children are more vulnerable. Cerebral malaria is associated with retinal whitening, which may be a useful clinical sign in distinguishing malaria from other causes of fever.

Species	Appearance	Periodicity	Liver persistent
Plasmodium vivax		tertian	yes
Plasmodium ovale		tertian	yes
Plasmodium falciparum		tertian	no
Plasmodium malariae		quartan	no

Severe malaria is almost exclusively caused by *P. falciparum* infection, and usually arises 6–14 days after infection. Consequences of severe malaria include coma and death if untreated—young children and pregnant women are especially vulnerable. Splenomegaly (enlarged spleen), severe headache, cerebral ischemia, hepatomegaly (enlarged liver), hypoglycemia, and hemoglobinuria with renal failure may occur. Renal failure is a feature of blackwater fever, where hemoglobin from lysed red blood cells leaks into the urine. Severe malaria can progress extremely rapidly and cause death within hours or days. In the most severe cases of the disease, fatality rates can exceed 20%, even with intensive care and treatment. In endemic areas, treatment is often less satisfactory and the overall fatality rate for all cases of malaria can be as high as one in ten. Over the longer term, developmental impairments have been documented in children who have suffered episodes of severe malaria.

Chronic malaria is seen in both *P. vivax* and *P. ovale*, but not in *P. falciparum*. Here, the disease can relapse months or years after exposure, due to the presence of latent parasites in the liver. Describing a case of malaria as cured by observing the disappearance of parasites from the bloodstream can, therefore, be deceptive. The longest incubation period reported for a *P. vivax* infection is 30 years. Approximately one in five of *P. vivax* malaria cases in temperate areas involve overwintering by hypnozoites (i.e., relapses begin the year after the mosquito bite).

Causes



A *Plasmodium* sporozoite traverses the cytoplasm of a mosquito midgut epithelial cell in this false-color electron micrograph.

Malaria parasites

Malaria parasites are members of the genus *Plasmodium* (phylum Apicomplexa). In humans malaria is caused by *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi*. *P. falciparum* is the most common cause of infection and is responsible for about 80% of all malaria cases, and is also responsible for about 90% of the deaths from malaria. Parasitic *Plasmodium* species also infect birds, reptiles, monkeys, chimpanzees and rodents. There have been documented human infections with several simian species of malaria, namely *P. knowlesi*, *P. inui*, *P. cynomolgi*, *P. simiovale*, *P. brazilianum*, *P. schwetzi* and *P. simium*; however, with the exception of *P. knowlesi*, these are mostly of limited public health importance.

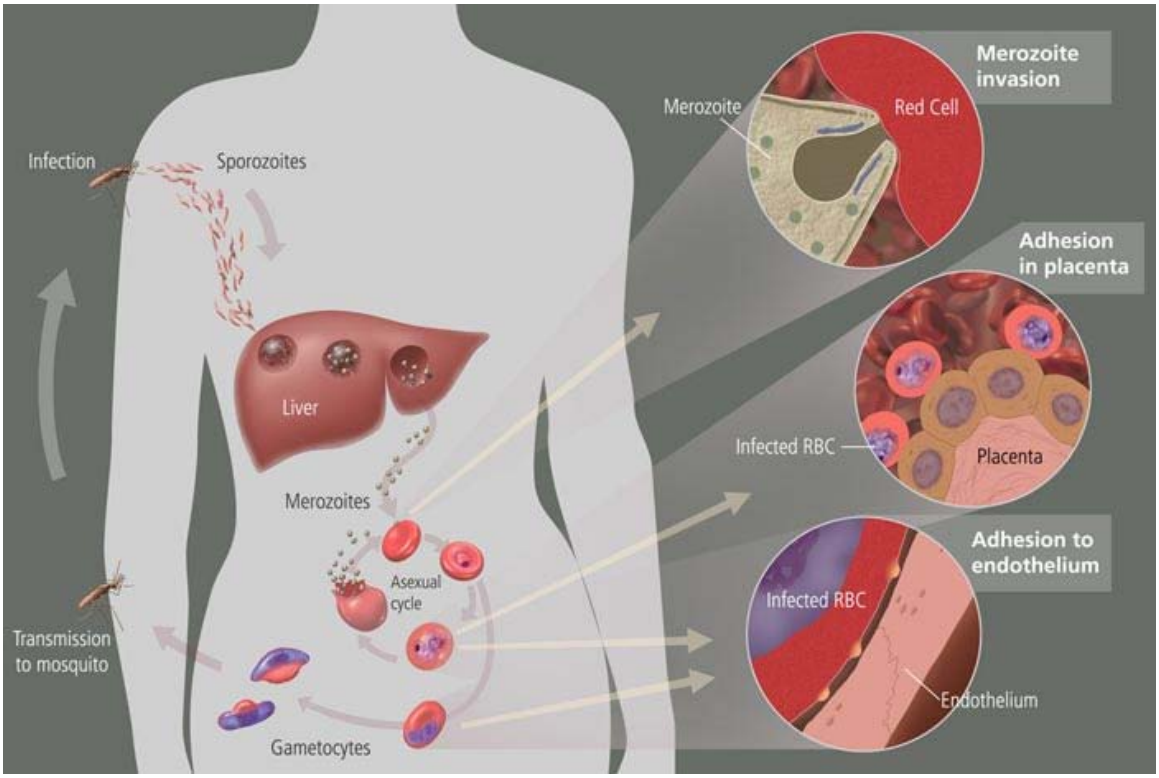
Malaria parasites contain apicoplasts, an organelle usually found in plants, complete with their own functioning genomes. These apicoplast are thought to have originated through the endosymbiosis of algae and play a crucial role in various aspects of parasite metabolism e.g. fatty acid bio-synthesis. To date, 466 proteins have been found to be produced by apicoplasts and these are now being looked at as possible targets for novel anti-malarial drugs.

Mosquito vectors and the *Plasmodium* life cycle

The parasite's primary (definitive) hosts and transmission vectors are female mosquitoes of the *Anopheles* genus, while humans and other vertebrates are secondary hosts. Young mosquitoes first ingest the malaria parasite by feeding on an infected human carrier and the infected *Anopheles* mosquitoes carry *Plasmodium* sporozoites in their salivary glands. A mosquito becomes infected when it takes a blood meal from an infected human. Once ingested, the parasite gametocytes taken up in the blood will further differentiate into male or female gametes and then fuse in the mosquito gut. This produces an ookinete that penetrates the gut lining and produces an oocyst in the gut wall. When the oocyst ruptures, it releases sporozoites that migrate through the mosquito's body to the salivary glands, where they are then ready to infect a new human host. This type of transmission is occasionally referred to as anterior station transfer. The sporozoites are injected into the skin, alongside saliva, when the mosquito takes a subsequent blood meal.

Only female mosquitoes feed on blood, thus males do not transmit the disease. The females of the *Anopheles* genus of mosquito prefer to feed at night. They usually start searching for a meal at dusk, and will continue throughout the night until taking a meal. Malaria parasites can also be transmitted by blood transfusions, although this is rare.

Pathogenesis



The life cycle of malaria parasites in the human body. A mosquito infects a person by taking a blood meal. First, sporozoites enter the bloodstream, and migrate to the liver. They infect liver cells (hepatocytes), where they multiply into merozoites, rupture the liver cells, and escape back into the bloodstream. Then, the merozoites infect red blood cells, where they develop into ring forms, trophozoites and schizonts which in turn produce further merozoites. Sexual forms (gametocytes) are also produced, which, if taken up by a mosquito, will infect the insect and continue the life cycle.

Malaria in humans develops via two phases: an exoerythrocytic and an erythrocytic phase. The exoerythrocytic phase involves infection of the hepatic system, or liver, whereas the erythrocytic phase involves infection of the erythrocytes, or red blood cells. When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver. Within 30 minutes of being introduced into the human host, the sporozoites infect hepatocytes, multiplying asexually and asymptomatically for a period of 6–15 days. Once in the liver, these organisms differentiate to yield thousands of merozoites, which, following rupture of their host cells, escape into the blood and infect red blood cells, thus beginning the erythrocytic stage of the life cycle. The parasite escapes from the liver undetected by wrapping itself in the cell membrane of the infected host liver cell.

Within the red blood cells, the parasites multiply further, again asexually, periodically breaking out of their hosts to invade fresh red blood cells. Several such amplification

cycles occur. Thus, classical descriptions of waves of fever arise from simultaneous waves of merozoites escaping and infecting red blood cells.

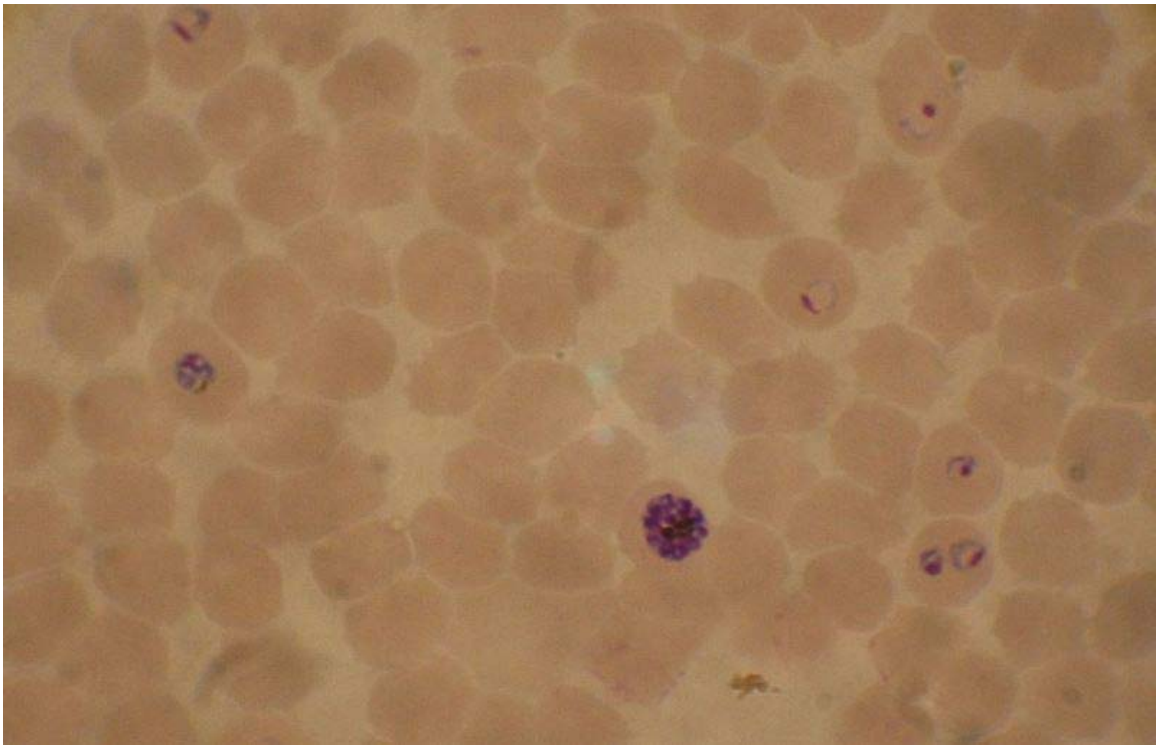
Some *P. vivax* and *P. ovale* sporozoites do not immediately develop into exoerythrocytic-phase merozoites, but instead produce hypnozoites that remain dormant for periods ranging from several months (6–12 months is typical) to as long as three years. After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in these two species of malaria.

The parasite is relatively protected from attack by the body's immune system because for most of its human life cycle it resides within the liver and blood cells and is relatively invisible to immune surveillance. However, circulating infected blood cells are destroyed in the spleen. To avoid this fate, the *P. falciparum* parasite displays adhesive proteins on the surface of the infected blood cells, causing the blood cells to stick to the walls of small blood vessels, thereby sequestering the parasite from passage through the general circulation and the spleen. This "stickiness" is the main factor giving rise to hemorrhagic complications of malaria. High endothelial venules (the smallest branches of the circulatory system) can be blocked by the attachment of masses of these infected red blood cells. The blockage of these vessels causes symptoms such as in placental and cerebral malaria. In cerebral malaria the sequestrated red blood cells can breach the blood brain barrier possibly leading to coma.

Although the red blood cell surface adhesive proteins (called PfEMP1, for *Plasmodium falciparum* erythrocyte membrane protein 1) are exposed to the immune system, they do not serve as good immune targets, because of their extreme diversity; there are at least 60 variations of the protein within a single parasite and effectively limitless versions within parasite populations. The parasite switches between a broad repertoire of PfEMP1 surface proteins, thus staying one step ahead of the pursuing immune system.

Some merozoites turn into male and female gametocytes. If a mosquito pierces the skin of an infected person, it potentially picks up gametocytes within the blood. Fertilization and sexual recombination of the parasite occurs in the mosquito's gut, thereby defining the mosquito as the definitive host of the disease. New sporozoites develop and travel to the mosquito's salivary gland, completing the cycle. Pregnant women are especially attractive to the mosquitoes, and malaria in pregnant women is an important cause of stillbirths, infant mortality and low birth weight, particularly in *P. falciparum* infection, but also in other species infection, such as *P. vivax*.

Diagnosis



Blood smear from a *P. falciparum* culture (K1 strain). Several red blood cells have ring stages inside them. Close to the center there is a schizont and on the left a trophozoite.

Since Charles Laveran first visualised the malaria parasite in blood in 1880, the mainstay of malaria diagnosis has been the microscopic examination of blood.

Fever and septic shock are commonly misdiagnosed as severe malaria in Africa, leading to a failure to treat other life-threatening illnesses. In malaria-endemic areas, parasitemia does not ensure a diagnosis of severe malaria, because parasitemia can be incidental to other concurrent disease. Recent investigations suggest that malarial retinopathy is better (collective sensitivity of 95% and specificity of 90%) than any other clinical or laboratory feature in distinguishing malarial from non-malarial coma.

Although blood is the sample most frequently used to make a diagnosis, both saliva and urine have been investigated as alternative, less invasive specimens.

Symptomatic diagnosis

Areas that cannot afford even simple laboratory diagnostic tests often use only a history of subjective fever as the indication to treat for malaria. Using Giemsa-stained blood smears from children in Malawi, one study showed that when clinical predictors (rectal temperature, nailbed pallor, and splenomegaly) were used as treatment indications, rather

than using only a history of subjective fevers, a correct diagnosis increased from 21% to 41% of cases, and unnecessary treatment for malaria was significantly decreased.

Microscopic examination of blood films

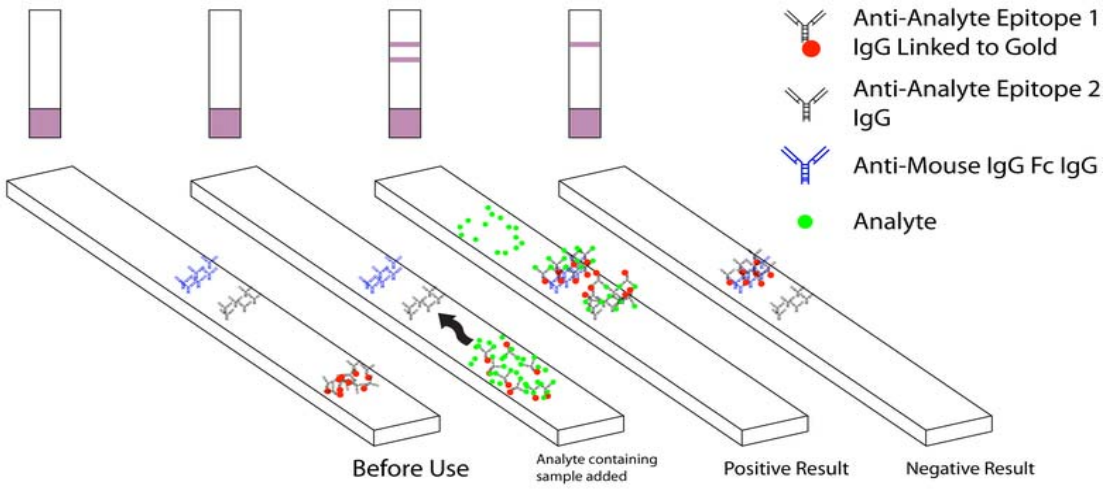
The most economic, preferred, and reliable diagnosis of malaria is microscopic examination of blood films because each of the four major parasite species has distinguishing characteristics. Two sorts of blood film are traditionally used. Thin films are similar to usual blood films and allow species identification because the parasite's appearance is best preserved in this preparation. Thick films allow the microscopist to screen a larger volume of blood and are about eleven times more sensitive than the thin film, so picking up low levels of infection is easier on the thick film, but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be much more difficult. With the pros and cons of both thick and thin smears taken into consideration, it is imperative to utilize both smears while attempting to make a definitive diagnosis.

From the thick film, an experienced microscopist can detect parasite levels (or parasitemia) down to as low as 0.0000001% of red blood cells. Diagnosis of species can be difficult because the early trophozoites ("ring form") of all four species look identical and it is never possible to diagnose species on the basis of a single ring form; species identification is always based on several trophozoites.

One important thing to note is that *P. malariae* and *P. knowlesi* (which is the most common cause of malaria in South-east Asia) look very similar under the microscope. However, *P. knowlesi* parasitemia increases very fast and causes more severe disease than *P. malariae*, so it is important to identify and treat infections quickly. Therefore modern methods such as PCR or monoclonal antibody panels that can distinguish between the two should be used in this part of the world.

Chapter 7

Malaria Antigen Detection Tests



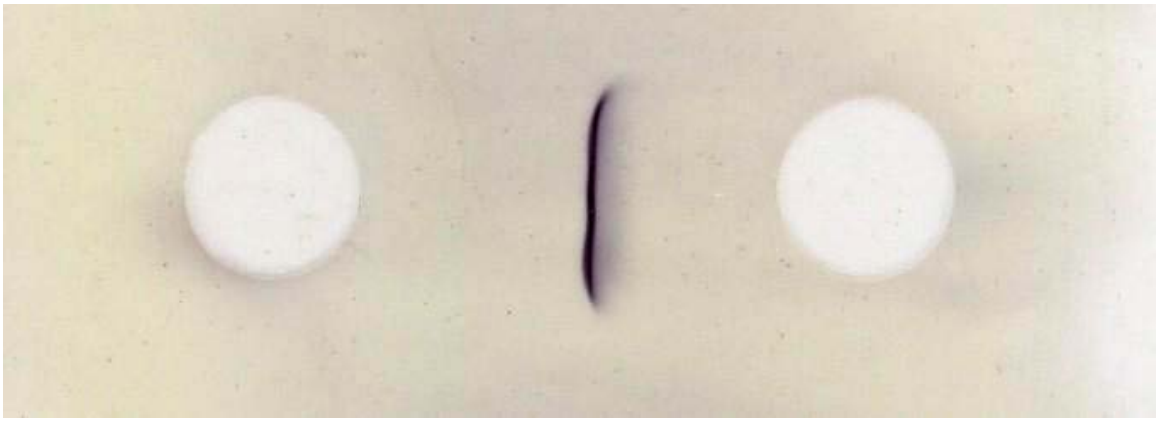
A schematic diagram of a dipstick

Malaria antigen detection tests are a group of commercially available tests that allow the rapid diagnosis of malaria by people who are not otherwise skilled in traditional laboratory techniques for diagnosing malaria or in situations where such equipment is not available. There are currently over 20 such tests commercially available (WHO product testing 2008). The first malaria antigen suitable as target for Rapid Diagnostic Tests (RDTs) was a soluble glycolytic enzyme Glutamate dehydrogenase. None of the rapid tests are currently as sensitive as a thick blood film, nor as cheap. A major drawback in the use of all current dipstick methods is that the result is essentially qualitative. In many endemic areas of tropical Africa, however, the quantitative assessment of parasitaemia is important, as a large percentage of the population will test positive in any qualitative assay.

Antigen-based Malaria Rapid Diagnostic Tests

Malaria is a curable disease if the patients have access to early diagnosis and prompt treatment. Antigen-based Rapid Diagnostic Tests (RDTs) have an important role at the periphery of health services capability because none of the rural clinics has the ability to diagnose malaria on-site due to a lack of microscopes and trained technicians to evaluate blood films. Furthermore, in regions where the disease is not endemic laboratory technologists have very limited experience in detecting and identifying malaria parasites. An ever increasing numbers of travelers from temperate areas each year visit tropical countries and many of them return with an malaria infection. The RDT tests are still regarded as complements to conventional microscopy but with some improvements it may well replace the microscope. The tests are simple and the procedure can be performed on the spot in field conditions. These tests use finger-stick or venous blood, the completed test takes a total of 15–20 minutes, and a laboratory is not needed. The threshold of detection by these rapid diagnostic tests is in the range of 100 parasites/µl of blood compared to 5 by thick film microscopy.

pGluDH



Plasmodium Glutamate dehydrogenase (pGluDH) precipitated by host antibodies

An accurate diagnosis is becoming more and more important, in view of the increasing resistance of *Plasmodium falciparum* and the high price of alternatives to chloroquine. The enzyme pGluDH does not occur in the host red blood cell and was recommended as marker enzyme for *Plasmodium* species by Picard-Maureau et al. in 1975. The malaria marker enzyme test is suitable for routine work and is now a standard test in most departments dealing with malaria. Presence of pGluDH is known to represent parasite viability and a rapid diagnostic test using pGluDH as antigen would have the ability to differentiate live from dead organisms. A complete RDT with pGluDH as antigen has been developed in China and is now undergoing clinical trials. GluDHs are ubiquitous enzymes that occupy an important branch-point between carbon and nitrogen metabolism. Both NAD [EC 1.4.1.2] and NADP dependend GluDH [EC 1.4.1.4] enzymes are present in *Plasmodia*; the NAD dependend GluDH is relatively unstable and not useful for diagnostic purposes. Glutamate dehydrogenase provides an oxidizable

carbon source used for the production of energy as well as a reduced electron carrier, NADH. Glutamate is a principal amino donor to other amino acids in subsequent transamination reactions. The multiple roles of glutamate in nitrogen balance make it a gateway between free ammonia and the amino groups of most amino acids. Its crystal structure is published. The GluDH activity in *P.vivax*, *P.ovale* and *P. malariae* has never been tested, but given the importance of GluDH as a branch point enzyme, every cell must have a high concentration of GluDH. It is well known that enzymes with a high molecular weight (like GluDH) have many isozymes, which allows strain differentiations (given the right monoclonal antibody). The host produces antibodies against the parasitic enzyme indicating a low sequence identity.

Histidine Rich Protein II

The histidine-rich protein II (HRP II) is a histidine - and alanine -rich, water-soluble protein, which is localized in several cell compartments including the parasite cytoplasm. The antigen is expressed only by *P. falciparum* trophozoites. HRP II from *P. falciparum* has been implicated in the biocrystallization of hemozoin, an inert, crystalline form of ferriprotoporphyrin IX (Fe(3+)-PPIX) produced by the parasite. A substantial amount of the HRP II is secreted by the parasite into the host bloodstream and the antigen can be detected in erythrocytes, serum, plasma, cerebrospinal fluid and even urine as a secreted water-soluble protein. These antigens persist in the circulating blood after the parasitaemia has cleared or has been greatly reduced. It generally takes around two weeks after successful treatment for HRP2-based tests to turn negative, but may take as long as one month, which compromises their value in the detection of active infection. False positive dipstick results were reported in patients with rheumatoid-factor-positive rheumatoid arthritis. Since HPR-2 is expressed only by *P. falciparum*, these tests will give negative results with samples containing only *P. vivax*, *P. ovale*, or *P. malariae*; many cases of non-falciparum malaria may therefore be misdiagnosed as malaria negative (some *P.falciparum* strains also don't have HRP II). The variability in the results of pHRP2-based RDTs is related to the variability in the target antigen.

pLDH

P.falciparum lactate dehydrogenase (pLDH) is a 33 kDa oxidoreductase [EC 1.1.1.27]. It is the last enzyme of the glycolytic pathway, essential for ATP generation and one o the most abundant enzymes expressed by *P.falciparum*. PLDH does not persist in the blood but clears about the same time as the parasites following successful treatment. The lack of antigen persistence after treatment makes the pLDH test useful in predicting treatment failure. In this respect, pLDH is similar to pGluDH. LDH from *P. vivax*, *P.malariae*, and *P.ovale* exhibit 90-92% identity to pLDH from *P.falciparum*.

pAldo

Fructose-bisphosphate aldolase [EC 4.1.2.13] catalyzes a key reaction in glycolysis and energy production and is produced by all four species. The *P.falciparum* aldolase is a 41 kDa protein and is 61-68% homologous to known eukaryotic aldolases. Its crystal

structure has been published. The presence of antibodies against p41 in the sera of human adults partially immune to malaria suggest that p41 is implicated in protective immune response against the parasite.

Chapter 8

Malaria Prophylaxis

Malaria prophylaxis is the prevention of malaria. Malaria is one of the oldest known pathogens, and began having a major impact on human survival about 10,000 years ago with the birth of agriculture. The development of virulence in the parasite has been demonstrated using genomic mapping of samples from this period, confirming the emergence of genes conferring a reduced risk of developing the malaria infection. References to the disease can be found in manuscripts from ancient Egypt, India and China, illustrating its wide geographical distribution. The first treatment identified is thought to be Quinine, one of four alkaloids from the bark of the Cinchona tree. Originally it was used by the tribes of Ecuador and Peru for treating fevers. Its role in treating malaria was recognised and recorded first by an Augustine monk from Lima, Peru in 1633. Seven years later the drug had reached Europe and was being used widely with the name 'the Jesuit's bark'. From this point onwards the use of Quinine and the public interest in malaria increased, although the compound was not isolated and identified as the active ingredient until 1820. By the mid-1880's the Dutch had grown vast plantations of cinchona trees and monopolised the world market.

Quinine remained the only available treatment for malaria until the early 1920's. During the First World War German scientists developed the first synthetic antimalarial compound – Atabrin and this was followed by Resochin and Sontochin derived from 4-aminoquinoline compounds. American troops, on capturing Tunisia during the Second World War, acquired, then altered the drugs to produce Chloroquine.

The development of new antimalarial drugs spurred the World Health Organization in 1955 to attempt a global malaria eradication program. This was successful in much of Brazil, the US and Egypt but ultimately failed elsewhere. Efforts to control malaria are still continuing, with the development of drug-resistant parasites presenting increasingly difficult problems.

Most adults from endemic areas have a degree of long-term infection, which tends to recur, and also possess partial immunity (resistance); the resistance reduces with time, and such adults may become susceptible to severe malaria if they have spent a significant amount of time in non-endemic areas. They are strongly recommended to take full precautions if they return to an endemic area.

Basic prevention

The ABCD of malaria prevention are:

- **A**wareness of risk;
- **B**ite prevention - Travelers to malarious areas are advised to wear long clothes that cover as much of the skin as possible. Exposed parts of the body should be treated with insect repellent. When sleeping, insecticide-impregnated bed nets should be used.
- **C**hemoprophylaxis; and
- rapid **D**iagnosis and treatment.

Recent improvements to the ABCD malaria prevention strategy have further enhanced its effectiveness in combating areas highly infected with the malaria parasite. Additional bite prevention measures include mosquito and insect repellents that can be directly applied to skin. This form of mosquito repellent is slowly replacing indoor residual spraying, which is shown to have high levels of toxicity by WHO. Further additions to preventative care are sanctions on blood transfusions. Once the malaria parasite enters the erythorocytic stage, it can adversely affect blood cells, making it possible to contract the parasite through infected blood.

Suppressive prophylaxis

Chloroquine, proguanil, mefloquine, and doxycycline are suppressive prophylactics. This means that they are only effective at killing the malaria parasite once it has entered the erythrocytic stage (blood stage) of its life cycle, and therefore have no effect until the liver stage is complete. That is why these prophylactics must continue to be taken for four weeks after leaving the area of risk.

Causal prophylaxis

Causal prophylactics target not only the blood stages of malaria, but the initial liver stage as well. This means that the user can stop taking the drug seven days after leaving the area of risk. Malarone and primaquine are the only causal prophylactics in current use.

Drug regimens

The following regimens are recommended by the WHO, UK HPA and CDC for prevention of P. falciparum infection

- doxycycline 100 mg once daily (started one day before travel, and continued for four weeks after returning);
- mefloquine 228 to 250 mg once weekly (started two-and-a-half weeks before travel, and continued for four weeks after returning);

- Malarone 1 tablet daily (started one day before travel, and continued for 1 week after returning).

In areas where chloroquine remains effective:

- chloroquine 300 to 310 mg once weekly, and proguanil 200 mg once daily (started one week before travel, and continued for four weeks after returning);

What regimen is appropriate depends on the person who is to take the medication as well as the country or region travelled to. This information is available from the UK HPA, WHO or CDC (links are given below). Doses depend also on what is available (e.g., in the US, mefloquine tablets contain 228 mg base, but 250 mg base in the UK). The data is constantly changing and no general advice is possible.

Doses given are appropriate for adults and children aged 12 and over.

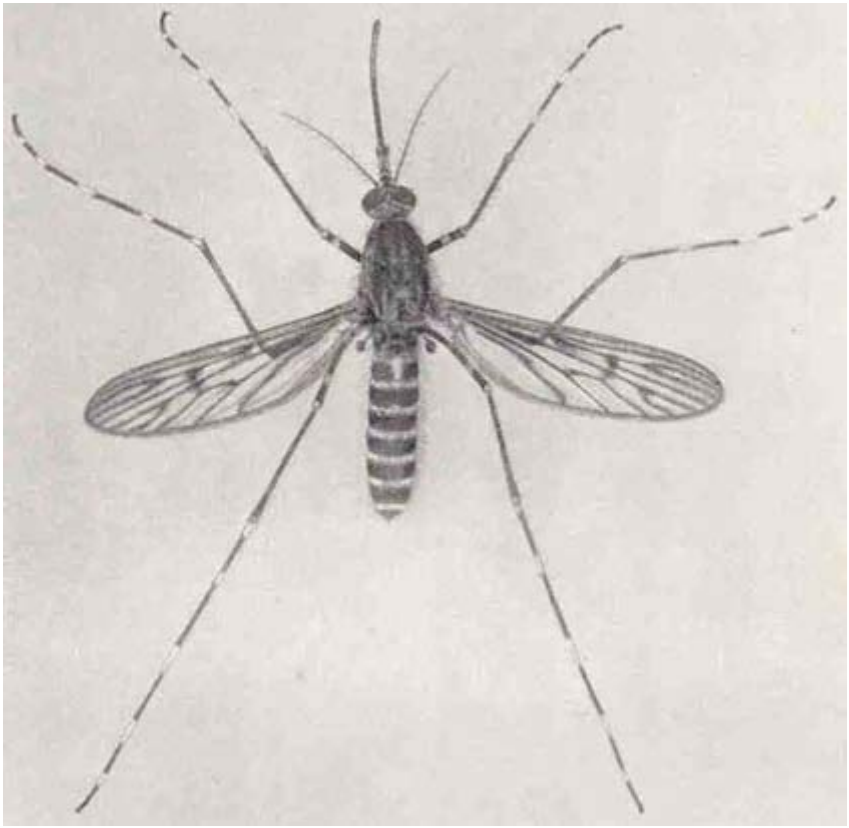
Other chemoprophylactic regimens that have been used on occasion:

- Dapsone 100 mg and pyrimethamine 12.5 mg once weekly (available as a combination tablet called Maloprim or Deltaprim): this combination is not routinely recommended because of the risk of agranulocytosis;
- Primaquine 30 mg once daily (started the day before travel, and continuing for seven days after returning): this regimen is not routinely recommended because of the need for G-6-PD testing prior to starting primaquine.
- Quinine sulfate 300 to 325 mg once daily: this regimen is effective but not routinely used because of the unpleasant side effects of quinine.

Prophylaxis against Plasmodium vivax requires a different approach given the long liver stage of this parasite. This is a highly specialist area.

Chapter 9

Mosquito Control



Mosquitos are generally considered annoying and may also transmit diseases, thus leading to a variety of human efforts to eradicate or reduce their presence.

Mosquito control manages the population of mosquitoes to reduce their damage to human health, economies, and enjoyment. Mosquito control is a vital public-health practice throughout the world and especially in the tropics because mosquitoes spread many diseases, such as malaria.

Mosquito-control operations are targeted against three different problems:

1. **Nuisance mosquitoes** bother people around homes or in parks and recreational areas;
2. **Economically important mosquitoes** reduce real estate values, adversely affect tourism and related business interests, or negatively impact livestock or poultry production;
3. **Public health** is the focus when mosquitoes are vectors, or transmitters, of infectious disease.

Disease organisms transmitted by mosquitoes include West Nile virus, Saint Louis encephalitis virus, Eastern equine encephalomyelitis virus, Everglades virus, Highlands J virus, La Crosse Encephalitis virus in the United States; dengue fever, yellow fever, Ilheus virus, and malaria in the American tropics; Rift Valley fever, *Wuchereria bancrofti*, Japanese Encephalitis, chikungunya and malaria in Africa and Asia; and Murray Valley encephalitis in Australia.

In the United States, states with sizable mosquito-control programs include California, Florida, New Jersey, Louisiana, Minnesota, Michigan, North Dakota, and Texas, among others.

General methods

Depending on the situation, source reduction, biocontrol, larviciding (control of larvae), or adulticiding (control of adults) may be used to manage mosquito populations. These techniques are accomplished using habitat modification, pesticide, biological-control agents, and trapping.

Monitoring mosquito populations

Adult mosquito populations may be monitored via landing rate counts, or mechanical traps. For landing rate counts, an inspector visits a set number of sites every day, counting the number of adult female mosquitoes that land on a part of the body, such as an arm or both legs, within a given time interval. Mechanical traps use a fan to blow adult mosquitoes into a collection bag that is taken back to the laboratory for analysis of catch. The mechanical traps use visual cues (light, black/white contrasts) or chemical attractants that are normally given off by mosquito hosts (e.g. carbon dioxide, ammonia, lactic acid, octenol) to attract adult female mosquitoes. These cues are often used in combination.

Monitoring larval mosquito populations involves collecting larvae from standing water with a dipper or a turkey baster. The habitat, approximate total number of larvae and pupae, and species are noted for each collection.

Monitoring larval mosquito populations is done by providing artificial breeding spots (ovitraps) Ovitrap and collecting and counting the developing larvae in fixed intervals.

Source reduction

Since many mosquitoes breed in standing water, source reduction can be as simple as emptying water from containers around the home. This is something that homeowners can accomplish. For example, homeowners can eliminate mosquito breeding grounds by removing unused plastic pools, old tires, or buckets; by clearing clogged gutters and repairing leaks around faucets; by regularly changing water in bird baths; and by filling or draining puddles, swampy areas, and tree stumps. Eliminating such mosquito breeding areas can be an extremely effective and permanent way to reduce mosquito populations without resorting to insecticides. However, this may not be possible in parts of the developing world where water cannot be readily replaced due to irregular water supply.

Open water marsh management (OWMM) involves the use of shallow ditches, about 4 feet (1.2 m) wide and 2 feet (0.61 m) deep, to create a network of water flow within marshes and to connect the marsh to a pond or canal. The network of ditches drains the mosquito habitat and lets in fish which will feed on mosquito larvae. This reduces the need for other control methods such as pesticides. Simply giving the predators access to the mosquito larvae can result in long-term mosquito control. Open-water marsh management is used on both the eastern and western coasts of the United States.

Rotational impoundment management (RIM) involves the use of large pumps and culverts with gates to control the water level within an impounded marsh. RIM allows mosquito control to occur while still permitting the marsh to function in a state as close to its natural condition as possible. Water is pumped into the marsh in the late spring and summer to prevent the female mosquito from laying her eggs on the soil. The marsh is allowed to drain in the fall, winter, and early spring. Gates in the culverts are used to permit fish, crustaceans, and other marsh organisms to enter and exit the marsh. RIM allows the mosquito-control goals to be met while at the same time reducing the need for pesticide use within the marsh. Rotational impoundment management is used to a great extent on the east coast of Florida.

Biocontrol

Gambusia affinis (Mosquitofish), a natural mosquito predator

Biological control or "biocontrol" is the use of natural enemies to manage mosquito populations. There are several types of biological control including the direct introduction of parasites, pathogens and predators to target mosquitoes. Effective biocontrol agents include predatory fish that feed on mosquito larvae such as mosquitofish (*Gambusia affinis*) and some cyprinids (carps and minnows) and killifish. Tilapia will also consume mosquito larvae.

Other predators include dragonfly naiads, which consume mosquito larvae in the breeding waters, and adult dragonflies, which eat adult mosquitoes. Some other biocontrol agents that have had lesser degrees of success include the predator mosquito *Toxorhynchites* and predator crustaceans—Mesocyclops copepods, nematodes, and fungi. Some public agencies also employ other predators such as birds, bats, lizards and frogs, but evidence of effectiveness of these agents is only anecdotal.

Like all animals, mosquitoes have their own set of diseases. Invertebrate pathologists study these diseases in the hope that some of them can be utilized for mosquito management. Microbial pathogens of mosquitoes include viruses, bacteria, fungi, protozoa, nematodes, and microsproidia (Davidson 1981, Jahn 1986)

Also used as biological control agent are the dead spores of varieties of the natural soil bacterium *Bacillus thuringiensis*, especially *Bt israelensis* (BTI). BTI is used to interfere in the digestion systems of larvae. It can be dispersed by hand or dropped by helicopter in large areas. BTI is no longer effective after the larvae turn into pupae, because they stop eating.

Integrated pest management (IPM) is the use of the most environmentally appropriate method or combination of methods to control pest populations. Typical mosquito-control programs using IPM first conduct larval and adult surveys, in order to determine the species composition, relative abundance, and seasonal distribution of adult and larval mosquitoes, and only then are the best and most effective methods of control utilized.

West Nile virus and Encephalitis are spread by mosquitoes and they continue to be a problem in the United States.

Larviciding

Control of larvae can be accomplished through use of contact poisons, growth regulators, surface films, stomach poisons (including bacterial agents), and biological agents such as fungi, nematodes, copepods, and fish. A chemical commonly used in the United States is methoprene, considered slightly toxic to larger animals, which mimics and interferes with natural growth hormones in mosquito larvae, preventing development. Methoprene is frequently distributed in time-release briquette form in breeding areas.

It is believed by some researchers that the larvae of *Anopheles gambiae* (important vectors of malaria) can survive for several days on moist mud, and that treatments should therefore include mud and soil several meters from puddles.

In 1958, The National Malaria Eradication Program implemented the wide-scale use of DDT for mosquito control.

Adulticiding

Control of adult mosquitoes is the most familiar aspect of mosquito control to most of the public. It is accomplished by ground-based applications or via aerial application of chemical pesticides. Generally modern mosquito-control programs in developed countries use low-volume applications of pesticides, although some programs may still use thermal fogging. DDT was formerly used throughout the world for large area mosquito control, but it is now banned in most developed countries. Controversially, DDT remains in common use in many developing countries, which claim that the public-health cost of switching to other control methods would exceed the harm caused by using DDT. It is sometimes approved for use only in specific, limited circumstances where it is most effective, such as application to walls.

The role of DDT in combating mosquitoes has been the subject of considerable controversy. While some argue that DDT deeply damages biodiversity, others argue that DDT is the most effective weapon in combatting mosquitoes and hence malaria. While some of this disagreement is based on differences in the extent to which disease control is valued as opposed to the value of biodiversity, there is also genuine disagreement amongst experts about the costs and benefits of using DDT. Moreover, DDT-resistant mosquitoes have started to increase in numbers, especially in tropics due to mutations, reducing the effectiveness of this chemical; these mutations can rapidly spread over vast areas if pesticides are applied indiscriminately (Chevillon *et al.* 1999).

Other methods

A light trap that attracts and captures mosquitos

A newer approach to killing mosquitoes in a non-toxic way is to use a device that burns propane, thus generating carbon dioxide, warmth, and water vapor. These three elements, often coupled with a chemical attractant heated in this process, draws the mosquitoes toward the propane flame, where they are then sucked into a net or holder where they collect.

Some newer mosquito traps or known mosquito attractants emit a plume of carbon dioxide together with other mosquito attractants such as sugary scents, lactic acid, octenol, warmth, water vapor and sounds. By mimicking a mammal's scent and outputs, the trap draws female mosquitoes toward it, where they are typically sucked into a net or holder by an electric fan where they are collected. According to the American Mosquito Control Association, "these devices will, indeed, trap and kill measurable numbers of mosquitoes," but their effectiveness in any particular case will depend on a number of factors such as the size and species of the mosquito population and the type and location of the breeding habitat. They are useful in specimen collection studies to determine the types of mosquitoes prevalent in an area but are typically far too inefficient to be useful in reducing mosquito populations.

The advantage of non-toxic methods of control is they can be used in Conservation Areas.

A traditional approach in control mosquito populations is the use of lethal Ovitrap by providing artificial breeding spots for the mosquitoes but destroying the developing larvae.

The latest approach is the automatic lethal ovitrap which works like a traditional ovitrap but automates all steps needed to provide the breeding spots and to destroy the developing larvae.

A fan can make enough turbulence to prevent mosquitoes in the small area. Due to their weak wings mosquitoes can not fly, land and therefore bite in presence of a even a light breeze.

Indoor residual spraying

Indoor residual spraying or **IRS** is the process of spraying the inside of dwellings with an insecticide to kill mosquitoes that spread malaria. A dilute solution of insecticide is sprayed on the inside walls of certain types of dwellings—those with walls made from porous materials such as mud or wood but not plaster as in city dwellings. Mosquitoes are killed or repelled by the spray, preventing the transmission of the disease. In 2008, 44 countries employed IRS as a malaria control strategy. Several pesticides have historically been used for IRS, the first and most well-known being DDT.

World Health Organization recommendations

The World Health Organization (WHO) recommends IRS as one of three primary means of malaria control, the others being use of insecticide treated bednets (ITNs) and prompt treatment of confirmed cases with artemisinin-based combination therapies (ACTs). While previously the WHO had recommended IRS only in areas of sporadic malaria transmission, in 2006 it began recommending IRS in areas of endemic, stable transmission as well.

According to the WHO:

> “ [N]ational governments should:
>
> 1. Introduce and/or scale up coverage of targeted IRS as a primary malaria control intervention in countries where available data indicates that it can be effective towards achieving malaria targets.
> 2. Take all necessary steps to ensure effective implementation of IRS interventions, including selecting the appropriate insecticide, spraying where and when necessary and sustaining a high level of coverage, and to ”

prevent unauthorized or un-recommended use of public health insecticides.
3. Strengthen the managerial capacity of national malaria control programmes and improve human, technical and financial resources for the timely delivery and high coverage of effective interventions including IRS, with adequate monitoring and evaluation.

Furthermore, for IRS to be effective:

1. There must be a high percentage of sprayable surfaces within each dwelling.
2. The vector (mosquitos) must feed or rest indoors.
3. The targeted vectors must be susceptible (i.e. not resistant) to the insecticide being sprayed.

The WHO further states that "insecticide susceptibility and vector behaviour; safety for humans and the environment; and efficacy and cost-effectiveness" are factors that must be considered when selecting an insecticide for IRS.

Approved insecticides

Currently, the WHO has approved twelve different insecticides for IRS.

Insecticide	Class	Recommended dosage of active ingredient (g/m^2)	Duration of effective action (months)	Estimated cost per house per 6 months (US$)	WHO toxicity rating
DDT	Organochlorine	1–2	>6	1.60	II
Fenitrothion	Organophosphate	2	3–6	14.80	II
Malathion	Organophosphate	2	2–3	8.20	III
Pirimiphos-methyl	Organophosphate	1–2	2–3		III
Propoxur	Carbamate	1–2	3–6	18.80	II
Bendiocarb	Carbamate	0.1–0.4	2–6	13.80	II
Alpha-cypermethrin	Pyrethroid	0.02–0.03	4–6		II
Cyfluthrin	Pyrethroid	0.02–0.05	3–6		II
Deltamethrin	Pyrethroid	0.02–0.025	3–6	1.60	II
Etofenprox	Pyrethroid	0.1–0.3	3–6		U
Lambda-cyhalothrin	Pyrethroid	0.02–0.03	3–6	8.60	III
Bifenthrin	Pyrethroid	0.025–0.05	3–6		II

Cost effectiveness and efficacy

According to 2010 Cochrane review, IRS is an effective strategy for reducing malaria incidence. It is about as effective using insecticide treated nets (ITN)s, though ITNs may be a more effective at reducing morbidity in some situations.

Few studies have directly compared the cost effectiveness of IRS directly with other methods of malaria control. A study from 2008 assessed the cost effectiveness of seven African anti-malaria campaigns: two IRS campaigns and five insecticide treated bednet (ITN) distribution campaigns. The authors found that on a cost-per-child-death-averted basis, all were about the same, but the ITN campaigns were slightly more cost effective.

With regard to the cost effectiveness of various pesticides vis-a-vis each other for IRS, historically DDT has been considered the most cost effective, mainly because it lasts longer than alternatives and therefore dwellings can be sprayed less frequently. Actual studies on cost effectiveness are, in fact, lacking and none have taken account the adverse health and environmental effects of DDT or its alternatives. The United Nations Environment Programme (UNEP) concluded in 2008 that "IRS with DDT remains affordable and effective in many situations but, with regard to the direct costs, the relative advantage of DDT vis-à-vis alternative insecticides seems to be diminishing. The contextual evidence base on cost-effectiveness needs strengthening, and the external costs of DDT use vis-à-vis alternative insecticides require a careful assessment."

Residents' opposition to IRS

For IRS to be effective, at least 80% of homes and barns in an area must be sprayed, and if enough residents refuse spraying, the effectiveness of the whole program can be jeopardized. Many residents resist spraying of DDT in particular. This is due to a variety of factors, including its smell and the stains it leaves on the walls. While that stain makes it easier to check whether the room has been sprayed, it causes some villagers to resist the spraying of their homes or to resurface the wall, which eliminates the residual insecticidal effect. Pyrethroid insecticides are reportedly more acceptable since they do not leave visible residues on the walls.

In addition, DDT is not suitable for this type of spraying in Western-style plastered or painted walls, only traditional dwellings with unpainted walls made of mud, sticks, dung, thatch, clay, or cement. As rural areas of South Africa become more prosperous, there is a shift towards Western style housing, leaving fewer homes suitable for DDT spraying, and necessitating the use of alternative insecticides.

Other villagers object to DDT spraying because it does not kill cockroaches or bedbugs; rather, it excites such pests making them more active, so that often the use of another insecticide is additionally required. Pyrethroids such as deltamethrin and lambdacyhalothrin, on the other hand, are more acceptable to residents because they kill

these nuisance insects as well as mosquitoes. DDT has also been known to kill beneficial insects, such as wasps that kill caterpillars that, unchecked, destroy thatched roofs.

As a result, Mozambique's chief of infectious disease control, Avertino Barreto, says that resistance to DDT spraying is "homegrown", not due to "pressure from environmentalists". "They only want us to use DDT on poor, rural black people," he says. "So whoever suggests DDT use, I say, 'Fine, I'll start spraying in your house first.'"

Use of DDT

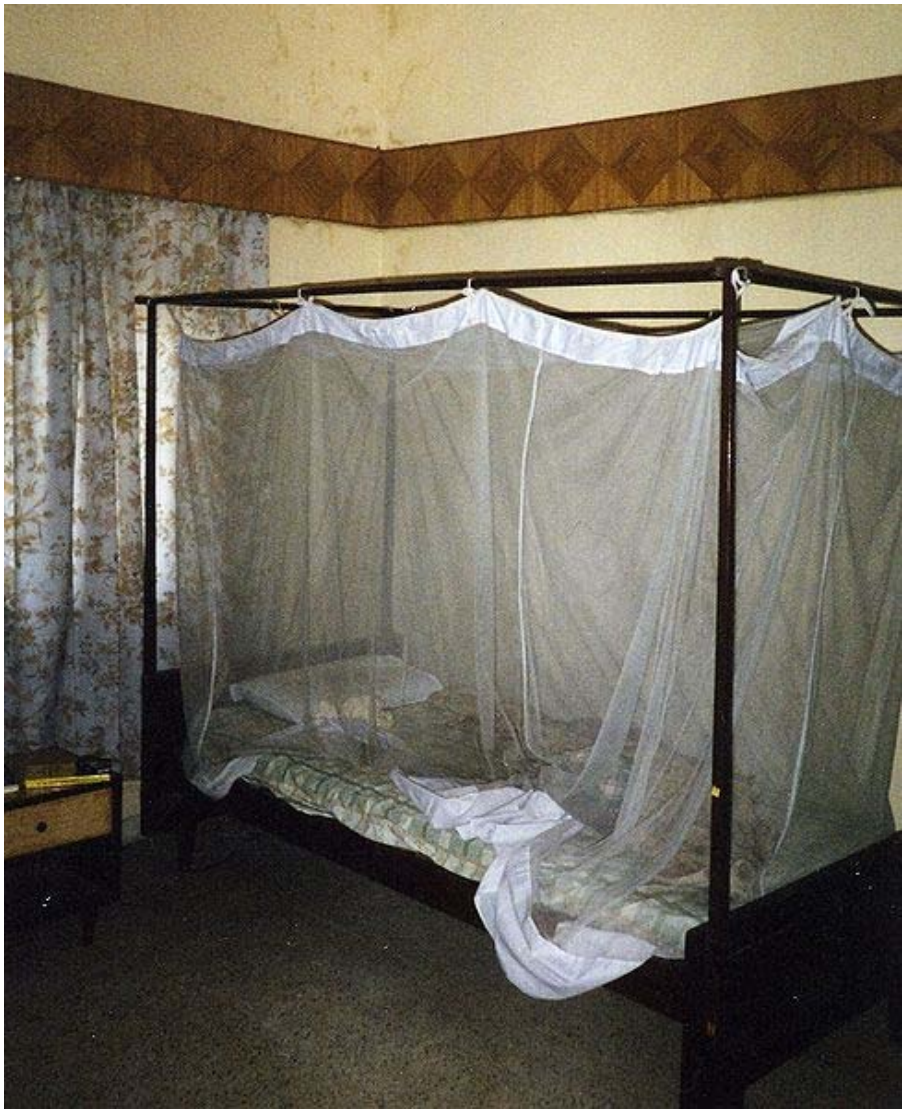
As discussed above, DDT is one 12 insecticides currently approved by the WHO for use in malaria control. The following table shows recent per country use of DDT for IRS. Unless otherwise noted, data for 2003–07 is from the 2008 Stockholm Convention/UNEP monograph on the current status of DDT, and 2008 data is from the WHO's World Malaria Report 2009. The World Malaria Report 2009 does not report the amount of DDT used in each country, only whether it is used or not. Accordingly, countries are listed as using 0 or "some" DDT.

Country	2003 use (tonnes)	2005 use (tonnes)	2007 use (tonnes)	2008 use	Notes
Botswana	0	0	0	0	Use suspended in 1997, plans to introduce in 2009
Cameroon	0	0	0	0	plan to introduce in 2009
China	0	0	n.a.	0	discontinued use in 2003
DR Congo	0	0	0	some	plan to reintroduce
Eritrea	13	15	15	some	
Ethiopia	272	398	371	some	Spraying stopped in 2010 because of resistance
Gambia	0	0	0	0	plan to introduce in 2008
Guyana	n.a.	n.a.	n.a.	some	listed in World Malaria Report 2008 as using DDT for IRS
India	4444	4253	3188	some	includes use for both malaria and leishmaniasis
Madagascar	45	0	0	0	plan to reintroduce in 2009
Malawi	0	0	0	0	plan to introduce in 2009
Mauritius	1	1	0	0	
Morocco	1	1	n.a.	0	
Mozambique	0	308	n.a.	some	reintroduced in 2005
Myanmar	1	1	n.a.	some	phasing out
Namibia	40	40	40	some	
North Korea	n.a.	n.a.	5	some	(an additional 155 tonnes is

					used in agriculture)
Papua New Guinea	n.a.	n.a.	n.a.	some	unknown amounts used
South Africa	54	62	66	some	reintroduced in 2000
Sudan	75	n.a.	0	0	
Swaziland	n.a.	8	8	some	
Uganda	0	0	0	some	IRS with DDT was briefly implemented in 2008
Zambia	7	26	22	some	reintroduced in 2000
Zimbabwe	0	108	12	some	reintroduced in 2004
Global Total	**4953**	**5219**	**3725**		

Mosquito net

Ceiling hung mosquito netting



Frame hung mosquito netting

Tent made of mosquito netting

Window with mosquito netting

A **mosquito net** offers protection against mosquitos, flies, and other insects, and thus against diseases such as malaria, dengue fever, yellow fever, and various forms of encephalitis, including the West Nile virus, if used properly and especially if treated with an insecticide, which can double effectiveness. The fine, see-through, mesh construction stops many insects from biting and disturbing the person using the net. The mesh is fine enough to exclude these insects, but it does not completely impede the flow of air.

History

Mosquito netting has a long history. Though use of the term dates from the mid-18th century, use of mosquito nets has been dated to prehistoric times. It is said that Cleopatra, Queen of Egypt, also slept under a mosquito net. Mosquito nets were used during the malaria-plagued construction of the Suez Canal.

Construction

Mosquito net can be made from cotton, polyethylene, polyester, or nylon. A mesh size of 1.2 mm stops mosquitoes, and smaller, such as 0.6 mm, stops other biting insects such as no-see-ums.

Usage

Mosquito nets are often used where malaria or other insect-borne diseases are common, especially as a tent-like covering over a bed. For effectiveness, it is important that the netting not have holes or gaps large enough to allow insects to enter. Because an insect can bite a person through the net, the net must not rest directly on the skin.

Mosquito netting can be hung over beds, from the ceiling or a frame, built into tents, or installed in windows and doors. When hung over beds, rectangular nets provide more room for sleeping without the danger of netting contacting skin, at which point mosquitoes may bite through untreated netting.

Insecticide treated nets

Mosquito nets treated with insecticides—known as insecticide treated nets (ITNs) -- were developed in the 1980s for malaria prevention. Insecticide-treated nets (ITN) are estimated to be twice as effective as untreated nets, and offer greater than 70% protection compared with no net. These nets are dip treated using a synthetic pyrethroid insecticide such as deltamethrin or permethrin which will double the protection over a non-treated net by killing and repelling mosquitoes.

The distribution of mosquito nets impregnated with insecticides such as permethrin or deltamethrin has been shown to be an extremely effective method of malaria prevention, and it is also one of the most cost-effective methods of prevention. These nets can often be obtained for around $2.50–$3.50 (2–3 euros) from the United Nations, the World Health Organization (WHO), and others. ITNs have been shown to be the most cost-effective prevention method against malaria and are part of WHO’s Millennium Development Goals (MDGs).

For maximum effectiveness, the nets should be re-impregnated with insecticide every six months. This process poses a significant logistical problem in rural areas. New technologies like Olyset or DawaPlus allow for production of long-lasting insecticidal mosquito nets (LLINs), which release insecticide for approximately 5 years, and cost about US$5.50. ITNs protect people sleeping under the net and simultaneously kill mosquitoes that contact the net. Some protection is also provided to others by this method, including people sleeping in the same room but not under the net.

While some experts argue that international organizations should distribute ITNs and LLINs to people for free in order to maximize coverage (since such a policy would reduces price barriers), others insist that cost-sharing between the international organization and recipients would led to greater usage of the net (arguing that people will value a good more if they pay for it). Additionally, proponents of cost-sharing argue that such a policy ensures that nets are efficiently allocated to those people who most need them (or are most vulnerable to infection). Through a "selection effect", they argue, those

people who most need the bed nets will choose to purchase them, while those less in need will opt out.

However, a randomized controlled trial study of ITNs uptake among pregnant women in Kenya, conducted by economists Pascaline Dupas and Jessica Cohen, found that cost-sharing does not necessarily increase the usage intensity of ITNs nor does it induce uptake by those most vulnerable to infection, as compared to a policy of free distribution. In some cases, cost-sharing can actually decrease demand for mosquito nets by erecting a price barrier. Dupas and Cohen's findings support the argument that free distribution of ITNs can be more effective than cost-sharing in both increasing coverage and saving lives. In a cost-effectiveness analysis, Dupas and Cohen note that *cost-sharing is at best marginally more cost-effective than free distribution, but free distribution leads to many more lives saved.*

The researchers base their conclusions about the cost-effectiveness of free distribution on the proven spillover benefits of increased ITN usage. When a large number of nets are distributed in one residential area, their chemical additives help reduce the number of mosquitoes in the environment. With fewer mosquitoes in the environment, the chances of malaria infection for recipients and non-recipients are significantly reduced. (In other words, the importance of the physical barrier effect of ITNs decreases relative to the positive externality effect of the nets in creating a mosquito-free environment when ITNs are highly concentrated in one residential cluster or community.)

Unfortunately, standard ITNs must be replaced or re-treated with insecticide after six washes and, therefore, are not seen as a convenient, effective long-term solution to the malaria problem. As a result, the mosquito netting and pesticide industries developed so-called long-lasting insecticidal mosquito nets (LLINs), which also use pyrethroid insecticides. There are two types of LLINs, one which is polyester netting and the insecticide is bound to the external surface of the netting fiber using a resin and another which incorporates the insecticide into a polyethylene fiber which then releases the insecticide slowly over 5 years. Both types can be washed at least 20 times but physical durability will vary, a survey carried out in Tanzania concluded that effective life of polyester nets was 2 to 3 years (Erlanger et al., 2004, Med Vet Entomol 18: 153-160), with polyethylene LLINs there is data to support > 5 years life with trials in showing nets which were still effective after 7 years (Tami, A et al. Malaria Journal 2004, 3:19). When calculating the cost of LLINs for large scale malaria prevention campaigns the cost should be divided by the number of years of expected life, hence a slightly more expensive net may be cheaper over time. In addition the logistical costs of replacing nets should be added to the calculation.

Scientific trials of ITNs

A review of 22 randomized controlled trials of ITNs found (for Plasmodium falciparum malaria) that ITNs can reduce deaths in children by one fifth and episodes of malaria by half.

More specifically, in areas of stable malaria "ITNs reduced the incidence of uncomplicated malarial episodes by 50% compared to no nets, and 39% compared to untreated nets" and in areas of unstable malaria "by 62% compared to no nets and 43% compared to untreated nets". As such the review calculated that for every 1000 children protected by ITNs, 5.5 lives would be saved each year.

Alternatives

Mosquito nets do reduce air flow to an extent and sleeping under a net is hotter than sleeping without one, which can be uncomfortable in tropical areas without air-conditioning.

One alternative for reducing mosquito bites is to use a fan to increase air flow, as mosquitoes prefer still air; however, this is far less effective and mosquito netting is preferable in areas with insect-borne diseases.

Another alternative is to apply an insect repellent to the skin; this also may be less effective (reducing rather than eliminating bites), more expensive, and may pose health risks with long-term use.

Chapter 10

Malaria Vaccine

Malaria vaccines are an area of intensive research, however, there is no effective vaccine that has been introduced into clinical practice. There is one candidate vaccine, known as RTS,S/AS01, which started Pivotal Phase III evaluation in May 2009 and is designed not for travellers but for children resident in malaria-endemic areas who suffer the burden of disease and death related to malaria.

The global burden of *P. falciparum* malaria increased through the 1990s due to drug-resistant parasites and insecticide-resistant mosquitoes; this is illustrated by re-emergence of the disease in areas that had been previously malaria-free. The first decade of the 21st century has seen reductions in morbidity and mortality in many settings. Though the reasons are not entirely clear, improving socioeconomic indices, deployment of artemisinin-combination drugs and insecticide-treated bednets are all likely to have contributed. There has been a major scaling-up in distribution of malaria control measures particularly since the advent of The Global Fund to Fight AIDS, Tuberculosis and Malaria. It is unclear what the future will hold for disease burden trends. If political will and funding is maintained, the disease burden could drop further; if as in the past funding lapses or clinically significant resistance develops to the main antimalarial drugs and insecticides used then the disease burden may rise again. Early evidence of resistance to artemisinins, the most important class of antimalarials, is now confirmed, having manifested as delayed parasite clearance times in the western region of Cambodia on the border with Thailand. This is also the region where resistance to earlier antimalarial drugs emerged and then subsequently spread throughout much of the world in the case of chloroquine. The Bill and Melinda Gates Foundation has launched a call for the aim of the malaria community to shift from sustained control to eradication. It is agreed that eradication is not possible with current tools and that research and development of new drugs, diagnostics, insecticides and a cost-effective deployable vaccine will be needed to facilitate eradication. There has been a great increase in funding for such research in the 21st century.

Vaccines are often the most cost-effective tools for public health. They have historically contributed to a reduction in the spread and burden of infectious diseases and have played the major part in previous elimination campaigns for smallpox and the ongoing polio and measles initiatives. Yet no effective vaccine for malaria has so far been developed. Despite this, researchers remain hopeful. Optimism is justified for several reasons, the first of these being that individuals who are exposed to the parasite in endemic countries

develop acquired immunity against disease and death. Such immunity does not however prevent malaria infection; immune individuals often harbour asymptomatic parasites in their blood. Additionally, research shows that if immunoglobulin is taken from immune adults, purified and then given to individuals that have no protective immunity, some protection can be gained. In addition to this, clinical and animal studies have shown that experimental vaccination has some degree of success when using attenuated sporozites and using the RTS,S/AS01 malaria vaccine candidate.

Considerations for vaccine development

The task of developing a preventative vaccine for malaria is a complex process. There are a number of considerations to be made concerning what strategy a potential vaccine should adopt.

The diversity of the parasite

P. falciparum has demonstrated the capability, through the development of multiple drug-resistance parasites, of evolutionary change. The *Plasmodium* species has a very high rate of replication, much higher than that actually needed to ensure transmission in the parasite's life cycle. This enables pharmaceutical treatments that are effective at reducing the reproduction rate, but not halting it, to exert a high selection pressure, thus favoring the development of resistance. The process of evolutionary change is one of the key considerations necessary when considering potential vaccine candidates. The development of resistance could cause a significant reduction in efficacy of any potential vaccine thus rendering useless a carefully developed and effective treatment.

Choosing to address the symptom or the source

There are two main types of immune response than could be elicited by the parasite. These are anti-parasitic immunity and anti-toxic immunity.

- "Anti-parasitic immunity" addresses the source; it consists of an antibody response (humoral immunity) and a cell-mediated immune response. Ideally a vaccine would enable the development of anti-plasmodial antibodies in addition to generating an elevated cell-mediated response. Potential antigens against which a vaccine could be targeted will be discussed in greater depth later. Antibodies are part of the specific immune response. They exert their effect by activating the complement cascade, stimulating phagocytic cells into endocytosis through adhesion to an external surface of the antigenic substances, thus 'marking' it as offensive. Humoral or cell-mediated immunity consists of many interlinking mechanisms that essentially aim to prevent infection entering the body (through external barriers or hostile internal environments) and then kill any micro-organisms or foreign particles that succeed in penetration. The cell-mediated component consists of many white blood cells (such as monocytes, neutrophils, macrophages, lymphocytes, basophils, mast cells, natural killer cells, and eosinophils) that target foreign bodies by a variety of different mechanisms. In the

case of malaria both systems would be targeted to attempt to increase the potential response generated, thus ensuring the maximum chance of preventing disease.

- "Anti-toxic immunity" addresses the symptoms; it refers to the suppression of the immune response associated with the production of factors that either induce symptoms or reduce the effect that any toxic by-products (of micro-organism presence) have on the development of disease. For example, it has been shown that Tumor necrosis factor-alpha has a central role in generating the symptoms experienced in severe *P. falciparum* malaria. Thus a therapeutic vaccine could target the production of TNF-a, preventing respiratory distress and cerebral symptoms. This approach has serious limitations as it would not reduce the parasitic load; rather it only reduces the associated pathology. As a result, there are substantial difficulties in evaluating efficacy in human trials.

Taking this information into consideration an ideal vaccine candidate would attempt to generate a more substantial cell-mediated and antibody response on parasite presentation. This would have the benefit of increasing the rate of parasite clearance, thus reducing the experienced symptoms and providing a level of consistent future immunity against the parasite.

Potential targets of a vaccine

By their very nature, parasites are more complex organisms than bacteria and viruses, with more complicated structures and life cycles. This presents problems in vaccine development but also increases the number of potential targets for a vaccine. These have been summarised into the life cycle stage and the antibodies that could potentially elicit an immune response.

The life cycle of the malaria parasite is particularly complex, presenting initial developmental problems. Despite the huge number of vaccines available at the current time, there are none that target parasitic infections. The distinct developmental stages involved in the life cycle present numerous opportunities for targeting antigens, thus potentially eliciting an immune response. Theoretically, each developmental stage could have a vaccine developed specifically to target the parasite. The initial stage in the life cycle, following inoculation, is a relatively short "pre-erythrocytic" or "hepatic" phase. A vaccine at this stage must have the ability to protect against sporozoites invading and possibly inhibiting the development of parasites in the hepatocytes (through inducing cytotoxic T-lymphocytes that can destroy the infected liver cells). However, if any sporozoites evaded the immune system they would then have the potential to be symptomatic and cause the clinical disease.

The second phase of the life cycle is the "erythrocytic" or blood phase. A vaccine here could prevent merozoite multiplication or the invasion of red blood cells. This approach is complicated by the lack of MHC molecule expression on the surface of erythrocytes. Instead, malarial antigens are expressed, and it is this towards which the antibodies could potentially be directed. Another approach would be to attempt to block the process of

erythrocyte adherence to blood vessel walls. It is thought that this process is accountable for much of the clinical syndrome associated with malarial infection; therefore a vaccine given during this stage would be therapeutic and hence administered during clinical episodes to prevent further deterioration. The last phase of the life cycle that has the potential to be targeted by a vaccine is the "sexual stage". This would not give any protective benefits to the individual inoculated but would prevent further transmission of the parasite by preventing the gametocytes from producing multiple sporozoites in the gut wall of the mosquito. It therefore would be used as part of a policy directed at eliminating the parasite from areas of low prevalence or to prevent the development and spread of vaccine-resistant parasites. This type of transmission-blocking vaccine is potentially very important. The evolution of resistance in the malaria parasite occurs very quickly, potentially making any vaccine redundant within a few generations. This approach to the prevention of spread is therefore essential.

Any vaccine produced would ideally have the ability to be of therapeutic value as well as preventing further transmission and is likely to consist of a combination of antigens from different phases of the parasite's development.

Sporozoite

- Abs that block hepatocyte invasion
- Abs that kill the sporozoite via complement fixation or opsonization

Infected hepatocyte

- CTL mediated lysis
- CD4+ help for the activation and differentiation of CTL
- Localized cytokine release by T cells or APCs
- ADCC or C' mediated lysis,this CD4+ is useful in phagocytic cell to bind the MHC 11

Asexual erythrocytic

- Localized cytokine release that directly kills infected erythrocyte or intracellular parasite
- Abs that agglutinate the merozoites before schizont rupture
- Abs that block merozoite invasion of RBCs
- Abs that kill iRBC via opsonization or phagocytotic mechanisms
- Abs engulfed with the merozoite at time of invasion which kill intraerythrocytic parasite
- Abs which agglutinate iRBCs and prevent cytoadherence by blocking receptor-ligand interactions (CD-36 is such a receptor)
- Abs which neutralize harmful soluble parasite toxins

Sexual erythrocytic

- Cytokines which kill gametocytes within the iRBC
- Abs that kill gametocytes within iRBC via C'
- Abs that interfere with fertilization
- Abs that inhibit transformation of the zygote into the ookinete
- Abs that block the egress of the ookinete from the mosquito midgut (Doolan and Hoffman)

When selecting the most suitable vaccine target the following considerations are made:

a) How accessible is the antigen to the immune system?

b) How susceptible is the antigen to evolutionary change?

c) How critical is the antigen to parasitic biological functions?

d) How likely is a protective response in animal models?

e) Does the antigen contain epitopes that are recognisable by HLA allele superfamilies?

f) How compatible is the antigen with other potential antigens?

Mix of antigenic components

Increasing the potential immunity generated against *Plasmodia* can be achieved by attempting to target multiple phases in the life cycle. This is additionally beneficial in reducing the possibility of resistant parasites developing. The use of multiple-parasite antigens can therefore have a synergistic or additive effect.

One of the most successful vaccine candidates currently in clinical trials consists of recombinant antigenic proteins to the circumsporozoite protein. (This is discussed in more detail below.)

Vaccine delivery system

The selection of an appropriate system is fundamental in all vaccine development, but especially so in the case of malaria. A vaccine targeting several antigens may require delivery to different areas and by different means in order to elicit an effective response. Some adjuvants can direct the vaccine to the specifically targeted cell type—e.g. the use of Hepatitis B virus in the RTS,S vaccine to target infected hepatocytes—but in other cases, particularly when using combined antigenic vaccines, this approach is very complex. Some methods that have been attempted include the use of two vaccines, one directed at generating a blood response and the other a liver-stage response. These two

vaccines could then be injected into two different sites, thus enabling the use of a more specific and potentially efficacious delivery system.

To increase, accelerate or modify the development of an immune response to a vaccine candidate it is often necessary to combine the antigenic substance to be delivered with an adjuvant or specialised delivery system. These terms are often used interchangeably in relation to vaccine development; however in most cases a distinction can be made. An adjuvant is typically thought of as a substance used in combination with the antigen to produce a more substantial and robust immune response than that elicited by the antigen alone. This is achieved through three mechanisms: by affecting the antigen delivery and presentation, by inducing the production of immunomodulatory cytokines, and by affecting the antigen presenting cells (APC). Adjuvants can consist of many different materials, from cell microparticles to other particulated delivery systems (e.g. liposomes).

Adjuvants are crucial in affecting the specificity and isotype of the necessary antibodies. They are thought to be able to potentiate the link between the innate and adaptive immune responses. Due to the diverse nature of substances that can potentially have this effect on the immune system, it is difficult to classify adjuvants into specific groups. In most circumstances they consist of easily identifiable components of micro-organisms that are recognised by the innate immune system cells. The role of delivery systems is primarily to direct the chosen adjuvant and antigen into target cells to attempt to increase the efficacy of the vaccine further, therefore acting synergistically with the adjuvant. There is increasing concern that the use of very potent adjuvants could precipitate autoimmune responses, making it imperative that the vaccine is focused on the target cells only. Specific delivery systems can reduce this risk by limiting the potential toxicity and systemic distribution of newly developed adjuvants. Studies into the efficacy of malaria vaccines developed to date have illustrated that the presence of an adjuvant is key in determining any protection gained against malaria. A large number of natural and synthetic adjuvants have been identified throughout the history of vaccine development. Options identified thus far for use combined with a malaria vaccine include mycobacterial cell walls, liposomes, monophosphoryl lipid A and squalene.

Vaccines developed up to now

The epidemiology of malaria varies enormously across the globe, and has led to the belief that it may be necessary to adopt very different vaccine development strategies to target the different populations. A Type 1 vaccine is suggested for those exposed mostly to P.falciparum malaria in sub-Saharan Africa, with the primary objective to reduce the number of severe malaria cases and deaths in infants and children exposed to high transmission rates. The Type 2 vaccine could be thought of as a ‘travellers’ vaccine’, aiming to prevent all cases of clinical symptoms in individuals with no previous exposure. This is another major public health problem, with malaria presenting as one of the most substantial threats to travellers’ health. Problems with the current available pharmaceutical therapies include costs, availability, adverse effects and contraindications, inconvenience and compliance many of which would be reduced or eliminated entirely if an effective (greater than 85-90%) vaccine was developed.

There are many antigens present throughout the parasite life cycle that potentially could act as targets for the vaccine. More than 30 of these are currently being researched by teams all over the world in the hope of identifying a combination that can elicit immunity in the inoculated individual. Some of the approaches involve surface expression of the antigen, inhibitory effects of specific antibodies on the life cycle and the protective effects through immunization or passive transfer of antibodies between an immune and a non-immune host. The majority of research into malarial vaccines has focused on the *Plasmodium falciparum* strain due to the high mortality caused by the parasite and the ease of a carrying out in vitro/in vivo studies. The earliest vaccines attempted to use the parasitic circumsporozoite (CS) protein. This is the most dominant surface antigen of the initial pre-erythrocytic phase. However, problems were encountered due to low efficacy, reactogenicity and low immunogenicity.

The first vaccine developed that has undergone field trials, is the SPf66, developed by Manuel Elkin Patarroyo in 1987. It presents a combination of antigens from the sporozoite (using CS repeats) and merozoite parasites. During phase I trials a 75% efficacy rate was demonstrated and the vaccine appeared to be well tolerated by subjects and immunogenic. The phase IIb and III trials were less promising, with the efficacy falling to between 38.8% and 60.2%. A trial was carried out in Tanzania in 1993 demonstrating the efficacy to be 31% after a year of follow up, however the most recent (though controversial) study was carried out in Gambia. It did not show any effect despite the relatively long trial periods and the number of studies carried out. It is still not known how the SPf66 vaccine confers immunity; it therefore remains an unlikely solution to malaria. The CSP was the next vaccine developed that initially appeared promising enough to undergo trials. It is also based on the circumsporoziote protein, but additionally has the recombinant (Asn-Ala-Pro15Asn-Val-Asp-Pro)2-Leu-Arg(R32LR) protein covalently bound to a purified *Pseudomonas aeruginosa* toxin (A9). However at an early stage a complete lack of protective immunity was demonstrated in those inoculated. The study group used in Kenya had an 82% incidence of parasitaemia whilst the control group only had an 89% incidence. The vaccine intended to cause an increased T-lymphocyte response in those exposed, this was also not observed.

The NYVAC-Pf7 multistage vaccine attempted to use different technology, incorporating seven *P.falciparum* antigenic genes. These came from a variety of stages during the life cycle. CSP and sporozoite surface protein 2 (called PfSSP2) were derived from the sporozoite phase. The liver stage antigen 1 (LSA1), three from the erythrocytic stage (merozoite surface protein 1, serine repeat antigen and AMA-1) and one sexual stage antigen (the 25-kDa Pfs25) were included. This was first investigated using Rhesus monkeys and produced encouraging results: 4 out of the 7 antigens produced specific antibody responses (CSP, PfSSP2, MSP1 and PFs25). Later trials in humans, despite demonstrating cellular immune responses in over 90% of the subjects had very poor antibody responses. Despite this following administration of the vaccine some candidates had complete protection when challenged with *P.falciparum*. This result has warranted ongoing trials.

In 1995 a field trial involving [NANP]19-5.1 proved to be very successful. Out of 194 children vaccinated none developed symptomatic malaria in the 12 week follow up period and only 8 failed to have higher levels of antibody present. The vaccine consists of the schizont export protein (5.1) and 19 repeats of the sporozoite surface protein [NANP]. Limitations of the technology exist as it contains only 20% peptide and has low levels of immunogenicity. It also does not contain any immunodominant T-cell epitopes.

RTS,S is the most recently developed recombinant vaccine. It consists of the *P. falciparum* cirumsporozoite protein from the pre-erythrocytic stage. The CSP antigen causes the production of antibodies capable of preventing the invasion of hepatocytes and additionally elicits a cellular response enabling the destruction of infected hepatocytes. The CSP vaccine presented problems in trials due to its poor immunogenicity. The RTS,S attempted to avoid these by fusing the protein with a surface antigen from Hepatitis B, hence creating a more potent and immunogenic vaccine. When tested in trials an emulsion of oil in water and the added adjuvants of monophosphoryl A and QS21 (SBAS2), the vaccine gave 7 out of 8 volunteers challenged with *P. falciparum* protective immunity.

Vaccine development strategies for the future

The development of a vaccine of therapeutic and protective benefit against the malaria parasite requires a novel approach as to date there are no vaccines available that effectively target a parasitic infection. The focus so far has been predominately on the use of sub-unit vaccines. The use of live, inactivated or attenuated whole parasites is not feasible and therefore antigenic particles, or subunits, from the parasite are isolated and tested for immunogenicity i.e. the ability to elicit an immune response. The majority of subunits tested have been discussed above and are frequently combined with adjuvants and specialised delivery systems to increase the very variable level of immune response. The most recent advances in the field of sub-unit vaccine development include the use of DNA vaccination. This approach involves removing sections of DNA from the parasitic genome and inserting the sequences into a vector, examples including plasmid genomes, attenuated DNA viral genomes, liposomes or proteoliposes, and other carrier complex molecules. When inoculated the plasmid or attenuated virus is endocytosed into a host cell, the DNA sequence is then incorporated into the host DNA and replicated by protein synthesis. The proteins then produced are expressed on the cell surface membrane of the ‘infected’ cell. These bind to the HLA molecules, priming T cells and therefore creating a population of memory T cells specific to the inoculated DNA sub-unit. This technique has been shown to produce a high rate of T cell response but poor level of antibody production. The efficacy of DNA vaccines can be assessed using an ELISPOT assay. The development of this method of testing for immune responses is extremely beneficial when examining the potential efficacy of a vaccine candidate and is hoped to enable critical analysis of the mechanisms that provide ‘partial’ protection, thus facilitating a greater understanding of vaccine technology. This approach of potentially allowing the modification of vaccine candidates to improve development techniques and further scientific understanding is known as ‘iterative development’. The advantage of DNA vaccines over classical attenuated vaccines are numerous and include being able to mimic

MHC class 1 CD8+ T cell specific responses that potentially could reduce some of the safety concerns associated with vaccine therapy and additionally provide a substantial reduction in production cost and due to the nature of DNA vaccines, increased ease of storage.

The most successful candidate developed to date is the RTS,S recombinant vaccine. The RTS,S/AS02A, one of the key vaccines produced using this technique, has been used in field trials in The Gambia. Three repeat doses were administered in the 6 months leading up to the period of highest malaria transmission. The vaccine efficacy was reported at approximately 71% (with 95% confidence intervals spanning from 46 to 85%) during the first 2 months of follow-up, but falling to 0% in the last 6 weeks in 250 male volunteers. Explanations for this are likely to be complex. On further analysis it was noticed that the majority of control subjects had become infected towards the end of the follow up period thus only the remaining (and therefore potentially more immune) subjects were included in the comparison against inoculated individuals, therefore as the efficacy of the vaccine decreased it was being tested against an increasingly immune cohort of controls, potentially explaining the massive decrease in protective immunity seen. Another reason to explain this is that an increase in the rate of malaria transmission during the final follow-up period occurred; this is particularly plausible and would coincide additionally with the suspected decrease in protection given by the last vaccine booster.