Blood and Tissue Protozoa
Medical Microbiology 201

Several pathogenic protozoa are found in the blood and tissues of humans (Table 1). In general these are transmitted by blood-feeding arthropod vectors. The exception is *Toxoplasma gondii* which is acquired by ingesting the infectious stages. These organisms exhibit relatively complex life cycles and in general the distribution of the vectors determine the distribution of the disease.

Table 1. Protozoa Found in Blood And Tissues of Humans		
Organism	**Vector**	**Disease and comments**
Trypanosoma gambiense and *T. rhodesiense*	Tse-tse fly	Acute febrile disease advancing to chronic disease of the CNS.
Trypansosma cruzi	Triatomine bugs	Long term chronic disease causing a cardiomyopathy.
Leishmania	Sand flies	A wide range of manifestations ranging from simple skin lesions to a systemic infection of the reticuloendothelial system.
Plasmodium	Mosquitoes	Causative agent of malaria.
Babesia	Ticks	A rare zoonotic infection.
Toxoplasma gondii	-	Normally a benign infection. Disease is usually associated with an immunosuppressed state.

Kinetoplastids

Kinetoplastids are a large and diverse group of flagellated protozoa characterized by a Geimsa-staining structure known as a kinetoplast. The kinetoplast is a specialized region of the mitochondria and the staining is due to mitochondrial. The kinetoplastids have more mitochondrial DNA than other eukaryotes resulting in a more intense staining. Three different kinetoplastids infect humans and cause disease: African trypanosomes, *Trypanosoma cruzi* and *Leishmania*.

African trypanosomiasis. African trypanosomes infecting humans are *T. rhodesiense* and *T. gambiense*. As the names imply, *T. gambiense* and *T. rhodesiense* are distinguished by their geographical distributions. *T. rhodesiense* is found in East Africa and *T. gambiense* is found in west and central Africa. The two species of human African trypanosomes differ in the virulence of the disease they cause as well as the ecology of transmission (Table 2). *T. rhodesiense* is a zoonotic infection which tends to produce a rapidly fulminating and acute disease, whereas *T. gambiense* is better adapted to the human host and usually causes a slowly progressing chronic disease. Acquisition of *T. rhodesiense* infections are usually sporadic infections associated with either occupational or recreational contact with wild animals (eg., safaris). *T. gambiense* is an endemic infection with both human-fly-human transmission as well as animal-fly-human transmission involving domestic animals.

African trypanosomes are transmitted by several species of the genus *Glossina*, commonly called the tse-tse. Trypanosomes are found in the saliva of the tse-tse and are transferred to the human host as the fly is taking a blood meal. Initially the trypanosomes are found in the blood and

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can result in irregular fever. This acute stage will last several weeks and the disease can spontaneously resolve or become asymptomatic. The infection can also become chronic characterized by invasion of the lymphatics and most notably the central nervous system. This progression from the acute stage to the chronic stage typically occurs over 6-12 months in the case of *T. gambiense.* Invasion of the CNS is characterized by a meningoencephalitis with various neurological impairments. If not treated, the nervous impairments tend to worsen until progressing to coma and death.

Table 2. Comparison of African Trypanosomes		
	T. rhodesiense	*T. gambiense*
tse-tse vector	*G. morsitans* group	*G. palpalis* group
ecology	dry bush or woodland	rainforest, riverine, lakes
transmission cycle	ungulate-fly-human	human-fly-human
		animal-fly-human
non-human reservoir	wild animals	domestic animals
epidemiology	sporadic, safaris	endemic, some epidemics
disease progression	rapid, often fatal	slow (~1 yr) acute → chronic
asymptomatic carriers	rare	common

Diagnosis of African trypanosomiasis is confirmed by detection of trypanosomes in blood, lymph node aspirates, or cerebral spinal fluid. Serological tests are also available. The drugs of choice for treatment in cases without CNS involvement are suramin and pentamidine. Treatment is generally effective and the prognosis is good. Melarsoprol is recommended if the CNS is involved. Melarsoprol, an arsenical based drug, exhibits a relatively high toxicity (4-12% of patients die from the drug treatment). Furthermore, the treatment course is long (minimum 10 days) and requires hospitalization, thus adding significantly to the costs. Eflornithine, an ornithine decarboxylase inhibitor, is an effective anti-trypanosomal drug that is being developed. It has been nicknamed the 'resurrection drug' because of its spectacular effect on comatose patients in late stage African sleeping sickness. However, it is expensive and the standard treatment is 14 consecutive daily injections.

American trypanosomiasis. *Trypanosoma cruzi* is the causative agent of Chagas' disease which is found in south and central America. *T. cruzi* is transmitted to the vertebrate host by triatomine bugs (also known as reduvids, kissing bugs, or assassin bugs). The triatomine bugs defecate while they are feeding and infective stages are eliminated with the feces. The trypanosomes gain access to the vertebrate host by entering through the bite wound, mucous membranes or hair follicles. The trypanosomes invade host cells and replicate as intracellular parasites.

Chagas disease is a complex process with a poorly understood pathophysiology. The disease exhibits 3 phases: acute, indeterminate (or latent), and chronic. The acute disease is characterized by active infection and symptoms may include fever, malaise, lymphadenopathy, hepatosplenomegaly, vomiting, diarrhea. However, most infected persons are asymptomatic or only have mild symptoms. Children are more likely to develop acute symptoms than adults. Following the acute phase is a 10-30 year latent period, characterized by seropositivity in the absence of detectable parasitemia and clinical symptoms. Chronic Chagas' disease is characterized by cardiomyopathy or megasyndromes. The cardiomyopathy may present with arrhythmias, conduction defects, cardiomegaly, throemboembolic events, or congestive heart failure. Megasyndromes are also a feature of chronic Chagas'

disease in certain geographical areas. The hollow viscera, especially colon and esophagus, are enlarged due to dilation.

Leishmaniasis. Several species of *Leishmania* infect humans and cause a myriad of syndromes (Table 3). Many *Leishmania* species are capable of infecting humans and most are due to zoonoses. There is strong tendency for a particular *Leishmania* species to exhibit a particular disease manifestation. However, any of the species is capable of causing any form of the disease. In addition, some species have a limited geographical range and certain types of disease tend to exhibit a geographical distribution.

Table 3. *Leishmania* Species Infecting Humans

Species	Clinical Manifestations	Geographical Distribution
L. mexicana complex	CL and rare cases of MCL and DCL.	Central America, northern and central S. America, very rare in southern USA
L. braziliensis complex	CL with some cases developing MCL later.	Central and South America
L. major	CL (wet)	northern and central Africa, Middle East, southern Asia
L. tropica	CL (dry) and rare cases of recidiva	Middle East and southern Asia
L. aethiopica	CL and rare cases of DCL	Ethiopia
L. donovani	VL with rare cases of post kala-azar CL	East Africa, sub-Sahara, southern Asia including India and Iran
L. infantum*	CL or VL according to strain	North Africa and southern Europe
L. chagasi*	VL	Foci in Brazil, Venezuela and Colombia. Isolated cases throughout S. America

*Possibly the same species introduced to the New World by the European colonists.

Leishmania is transmitted by small biting insects known as sandflies. Two genera of sandflies are implicated in human transmission: *Phlebotomus* in the old world (Asia, Africa, middle east, central Asia, Meditarania) and *Lutzomyia* in the new world (south and central America). As the sandfly feeds, the parasites are delivered into bite wound of the vertebrate host and phagocytosed by macrophages where they undergo multiple rounds of binary fission. The infected macrophage lyses and releases the parasites which are taken up by other macrophages, thus reinitiating the replicative cycle. This infection cycle can remain localized to the site of infection and produce a simple cutaneous disease, or metastasize and produce additional cutaneous and/or mucocutaneous lesions, or become a systemic infection of the reticulo-endothelial system and produce a visceral disease.

Cutaneous leishmaniasis (CL) is the most common manifestation of the disease. These are generally benign self-healing lesions that are painless and non-pruritic and take weeks to months to heal. The lesions can be either nodular or ulcerated. Ulcerated lesions tend to have a raised border. Diffuse cutaneous leishmaniasis (DCL) and leishmaniasis recidivans are two rare manifestations of cutaneous leishmaniasis. DCL is characterized by disseminated nodular lesions that resemble lepromatous leprosy. Recivida is a chronic recurrence of nodular lesions or a rash characterized by hypersensitivity. Neither DCL nor recidiva are easily cured

A small proportion of patients with simple cutaneous leishmaniasis will develop mucocutaneous leishmaniasis (MCL). This manifestation is primarily due to members of the *L. braziliensis* complex. The infected macrophages metastasize via the blood stream or lymphatics, particularly to the mucosae of the nose and mouth. The expression of this form of the disease can occur several years after the primary cutaneous lesion. This disease will generally continue to progress and can lead to severe pathology and deformity if not treated.

Visceral leishmaniasis (VL) is the most serious form of the disease and can be fatal if untreated. It is a generalized infection of the reticuloendothelial system (RES) involving the spleen, liver, bone marrow and lymph nodes. The disease is characterized by fever, spleno- and hepato-megaly, and enlarged lymph nodes and tonsils. Patients often exhibit a wasting syndrome despite good appetite as the disease progresses.

Definitive diagnosis depends on detecting or culturing the parasites from clinical specimens (eg., skin lesions or bone marrow). Immuno-diagnostic methods include a delayed hypersensitivity skin test or serological tests. The skin test is used in cases of suspected cutaneous leishmaniasis whereas serological tests are usually used in suspected visceral cases.

Pentavalent antimonials are generally the first line of treatment. These drugs have been used for more than 50 years and are generally effective. However, they require parenteral administration and a long duration of therapy. Toxic side effects are also common. In addition, treatment failures have been observed over recent years suggesting the emergence of drug resistance. Amphotericin B is an alternative to the pentavalent antimonials. It is effective, but does exhibit toxicity and also requires parenteral administration and a long duration of treatment. It is also more expensive than the pentavalent antimonials. An oral drug for the treatment of visceral leishmaniasis has recently been registered in India. This new drug, miltefosine (Impavido®), is highly effective, less toxic, easily to administer, and will probably cost less than current therapies since hospitalization will not be required.

Malaria

Malaria has been and still is the cause of much human morbidity and mortality. Although the disease has been eradicated in most temperate zones, it continues to be endemic throughout much of the tropics and subtropics. Forty percent of the world's population lives in endemic areas. Epidemics have devastated large populations and malaria poses a serious barrier to economic progress in many developing countries. There are an estimated 300-500 million cases of clinical disease per year with 1.5-2.7 million deaths.

The causative agents of malaria are members of the genus *Plasmodium*. *Plasmodium* species exhibit a heteroxenous life cycle involving a vertebrate host and a mosquito vector. Vertebrate hosts include: reptiles, birds, rodents, monkeys and humans. *Plasmodium* species are host specific and there are no zoonoses. Four distinct species infected humans: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The species differ in regards to their morphology, details of their life cycles, and their clinical manifestations.

Life cycle. Human *Plasmodium* species are transmitted by anopheline mosquitoes. The infection is initiated when sporozoites are injected with the saliva of a feeding mosquito.

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Sporozoites are carried by the circulatory system to the liver and invade hepatocytes. The intracellular parasite undergoes an asexual replication known as exoerythro-cytic schizogony within the hepatocyte. Exoerythrocytic schizogony culminates in the production of merozoites which are released into the bloodstream. A proportion of the liver-stage parasites from *P. vivax* and *P. ovale* go through a dormant period instead of immediately undergoing asexual replicatation. These hypnozoites will reactivate several weeks to months (or years) after the primary infection and are responsible for relapses.
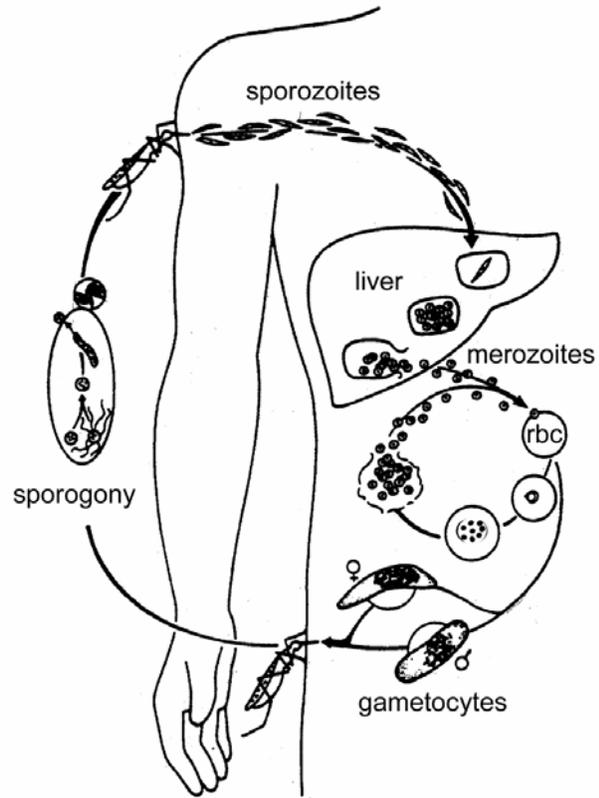
Merozoites invade erythrocytes and undergo a trophic period in which the para-site enlarges. The early trophozoite is often referred to as 'ring form' because of its mor-phology. Trophozoite enlargement is accom-panied by an active metabolism including the ingestion of host cytoplasm and the pro-teolysis of hemoglobin into amino acids. At the end of the trophic period the parasite undergoes multiple rounds of nuclear division without cytokinesis resulting is a schizont. Merozoites bud from the mature schizont and are released following rupture of the infected erythrocyte. Invasion of erythrocytes reinitiates another round of the blood-stage replicative cycle.

As an alternative to the asexual replicative cycle, the parasite can differentiate into sexual forms known as macro- and microgametocytes. Ingestion of gametocytes by the mosquito vector induces gametogenesis (i.e., the production of gametes) and escape from the host erythrocyte. Microgametes, formed by a process known as exflagellation, are flagellated forms which will fertilize the macrogamete leading to a zygote. The zygote develops into a motile ookinete which penetrates the gut epithelial cells and develops into an oocyst. The oocyst undergoes multiple rounds of asexual replication resulting in the production of sporozoites. Rupture of the mature oocyst releases the sporozoites into the hemocoel (i.e., body cavity) of the mosquito. The sporozoites migrate to and invade the salivary glands, thus completing the life cycle.

Pathology and clinical symptoms. The pathology and clinical manifestations associated with malaria are almost exclusively due to the asexual erythrocytic stage parasites. *Plasmodium* infection causes an acute febrile illness which is most notable for its periodic fever paroxysms occurring at either 48 or 72 hour intervals. These intermittent fever paroxyms are due to the synchronous lysis of the infected erythrocytes. *P. malariae* exhibits a 72 hour periodicity, whereas the other three species exhibit 48 hour cycles. *P. falciparum* often exhibits a continuous fever rather than the periodic paroxysms. The malarial paroxysm will usually last 4-8 hours and begins with a sudden onset of chills in which the patient experiences an intense feeling of cold despite having an elevated temperature. This is often referred to as the cold stage and is

characterized by a vigorous shivering. Immediately following this cold stage is the hot stage. The patient feels an intense heat accompanied by severe headache. Fatigue, dizziness, anorexia, myalgia, and nausea will often be associated with the hot stage. Next a period of profuse sweating will ensue and the fever will start to decline. The patient is exhausted and weak and will usually fall asleep. Upon awakening the patient usually feels well, other than being tired, and does not exhibit symptoms until the onset of the next paroxysm.
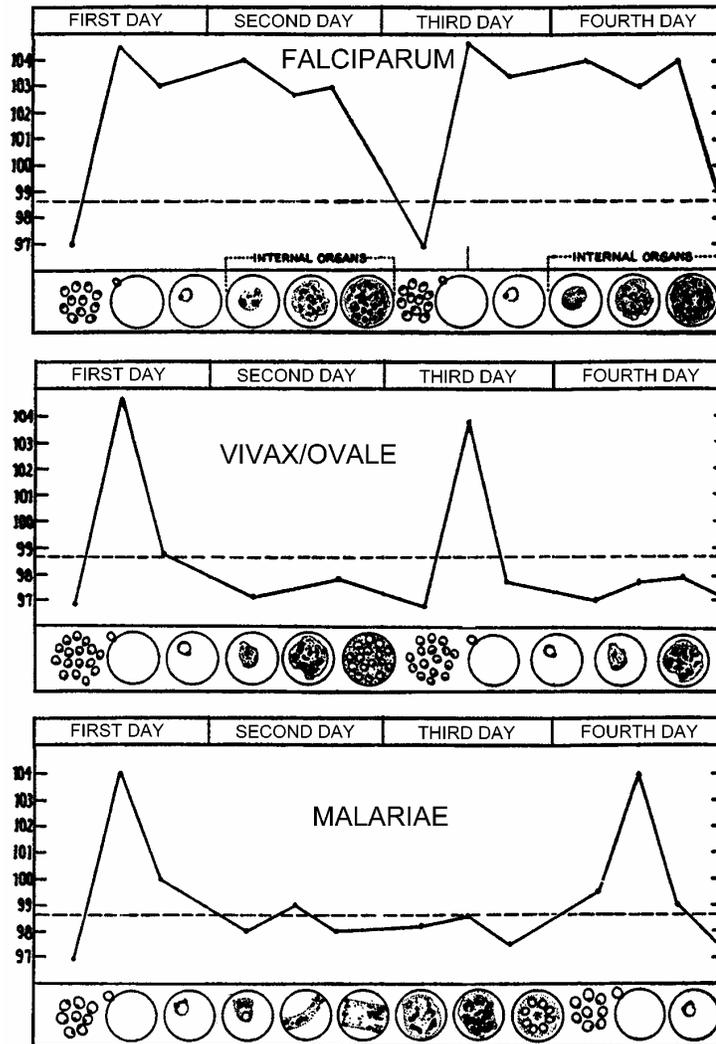
P. falciparum also is responsible for more morbidity and mortality than the other species (Table 4). This increase virulence is due in large part to the higher parasitemias associated with *P. falciparum* infections. In addition, complications are associated with *P. falciparum* because of the sequestration of the trophozoite- and schizont-infected erythrocytes in the deep tissues. This sequestration is due to the cytoadherence of infected erythrocytes to endothelial cells. Cytoadherence appears to be mediated by the electron-dense protuberances on the surface of the infected erythrocyte. These electron-dense 'knobs' are expressed during the trophozoite and schizont stages and are formed as a result of parasite proteins exported to the erythrocyte membrane. Electron

microscopy also shows that the knobs are contact points between the infected erythrocyte and the endothelial cell.
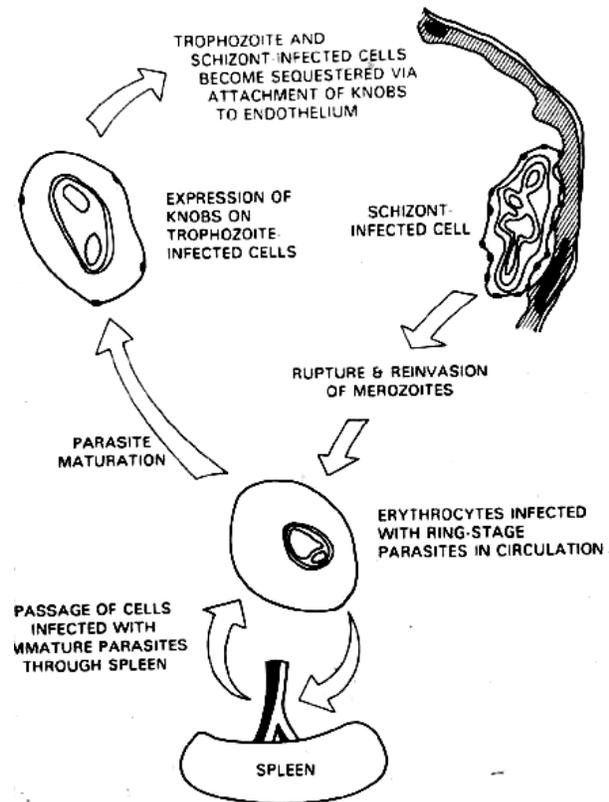
Table 4. Disease Severity and Duration

	vivax	ovale	malariae	falciparum
Incubation Period (days)	8-27	8-27	16->40	6-25
Severity of Initial Paroxysms	moderate to severe	mild	mild to moderate	severe
Average Parasitemia (per mm^3)	20,000	9,000	6,000	50,000-500,000
Maximum Parasitemia (per mm^3)	50,000	30,000	20,000	2,500,000
Typical Symptom Duration (untreated)	3-8 weeks	2-3 weeks	3-24 weeks	2-3 weeks
Maximum Infection Duration (untreated)	5-8 years*	12-20 months*	20-50 years	6-17 months
Anemia	++	+	++	++++
Other Complications			renal	cerebral**

Incubation period defined as time from sporozoite infection until appearance of symptoms. *Includes relapses (≠ recrudescence) due to dormant 'hypnozoite' stage in liver. **Other organs in addition to the brain are also affected in severe malaria.

This sequestration provides several advantages for the parasite. The major advantage is the avoidance of the spleen and the subsequent elimination of infected erythrocytes. In addition, the low oxygen tensions in the deep tissues may provide a better metabolic environment. The cytoadherence in the small capillaries may also permit a more efficient invasion of erythrocytes by merozoites when they are release.

This sequestration is also believed to contribute to cerebral malaria. Cerebral malaria is characterized by an impaired consciousness. The presenting symptoms are severe headache followed by drowsiness, confusion, and ultimately coma. Convulsions are also frequently associated with cerebral malaria. These neurological manifestations are believed to be due to the sequestration of the infected erythrocytes in the cerebral microvasculature. The sequestered infected erythrocytes can lead to a blockage of

TROPHOZOITE AND SCHIZONT-INFECTED CELLS BECOME SEQUESTERED VIA ATTACHMENT OF KNOBS TO ENDOTHELIUM

EXPRESSION OF KNOBS ON TROPHOZOITE-INFECTED CELLS

SCHIZONT-INFECTED CELL

RUPTURE & REINVASION OF MEROZOITES

PARASITE MATURATION

ERYTHROCYTES INFECTED WITH RING-STAGE PARASITES IN CIRCULATION

PASSAGE OF CELLS INFECTED WITH IMMATURE PARASITES THROUGH SPLEEN

SPLEEN

the capillaries leading to hypoxia. In addition, the actively metabolizing parasite can deplete the local glucose levels and produce a lactic acidosis. Proinflammatory cytokines, such as tumor necrosis factor-α, may also participate in the pathophysiology.

Diagnosis and treatment. Malaria is suspected in persons with a history of being in an endemic area and presenting symptoms consistent with malaria such as chills, fever, headache and malaise. These symptoms, especially in the early stages of the infection, are non-specific and often described as flu-like. As the disease progresses, the patient may exhibit an enlarged spleen and/or liver and anemia. Diagnosis is confirmed by detection of parasites in the blood. Thick blood smears are generally superior for the detection of parasites, whereas thin smears are preferable for species identification. If parasites are not found on the first blood smear it is recommended to make additional smears every 6-12 hours for as long as 48 hours. A tentative diagnosis of *P. falciparum* (numerous and exclusively ring stages) could constitute a medical emergency, especially in a non-immune person. Dipsticks based on antigen detection are also available.

Several antimalarial drugs are available. Many factors are involved in deciding the best treatment for malaria. These factors include the parasite species, the severity of disease and complications, the patient's age and immune status, the parasite's susceptibility to the drugs (i.e., drug resistance), and the cost and availability of drugs. Therefore, the exact recommendations will often vary according to geographical region. In addition, the various drugs act differentially on the different life cycle stages (Table 5).

Table 5. Selected Anti-Malarials	
Drug Action	**Drugs**
Fast-acting blood schizontocide	choloroquine (+ other 4-aminoquinolines), quinine, quinidine, mefloquine, halofantrine, antifolates (pyrimethamine, proquanil, sulfadoxine, dapsone), artemisinin derivatives (quinhaosu)
Slow-acting blood schizontocide	doxycycline (other tetracycline antibiotics)
Blood + mild tissue schizontocide	proquanil, pyrimethamine, tetracyclines
Anti-relapsing	primaquine
Gametocidal	primaquine, 4-aminoquinolines (limited?)
Combinations	Fansidar (pyrimethamine + sulfadoxine), Maloprim (pyrimethamine + dapsone), Malarone (atovaquone + proquanil)

Fast-acting blood schizontocides, which act upon the parasite within erythrocytes, are used to treat acute infections and to quickly relieve the clinical symptoms. Chloroquine is generally the recommended treatment for patients with *P. vivax*, *P. ovale*, *P. malariae*, and uncomplicated chloroquine-sensitive *P. falciparum* infections. Chloroquine is safe and usually well tolerated. Patients infected with either *P. vivax* or *P. ovale*, and that are not at a high risk for reinfection, should also be treated with primaquine. Primaquine is effective against the liver stage of the parasite, including hypnozoites, and therefore may prevent future relapses.

The efficacy of chloroquine is greatly diminished by the wide spread chloroquine resistance of *P. falciparum* and the emergence of chloroquine-resistant *P. vivax*. If chloroquine therapy is not effective, or if in an area with chloroquine-resistant malaria, common alternative treatments include: mefloquine, quinine in combination with doxycycline, or Fansidar. Deriva-

tives of artemisinin (artesunate and artemether) are increasingly used in Asia and Africa. These drugs were originally derived from the wormwood plant (*Artemesia annua*) and have been used for a long time in China as an herbal tea called quinhaosu to treat febrile illnesses.

Severe, or complicated, falciparum malaria is a serious disease with a high mortality rate and requires urgent treatment. Typically treatment requires parenteral drug administration (i.e., injections) since patients are often comatose or cannot take the drugs orally. Parenteral formulations are available for chloroquine, quinine, quinidine and artemisinin derivatives. Artemisinin suppositories have also been developed. Patients need to be continuously monitored for hematocrit, parasitemia, hydration levels, and signs of drug toxicity and other complications. A switch to oral administration should be made as soon as the patient is able.

Mark F. Wiser, Ph.D.
Department of Tropical Medicine
wiser@tulane.edu
http://www.tulane.edu/~wiser/T2.html