Blood Parasite Identification

The BP-11 challenge is a photopage of a 30-year-old from Tanzania with a history of malaria. The specimen contained *Mansonella perstans*. A response of "Microfilaria-*Mansonella* sp.", "Microfilaria, NOS referred for identification", or "Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification" was considered satisfactory.

Table 1. Parasite Identification

Total Responses	Referees (55)		Participants (410)	
Identification	**N**	**%**	**N**	**%**
Microfilaria-*Mansonella* sp.	52	94.5	388	94.6
Unacceptable				
Microfilaria-*Brugia*	2	3.6	10	2.4
Microfilaria-*Loa loa*	1	1.8	6	1.5

Table 2. Parasite Screen

Total Responses	Referees (61)		Participants (905)	
Identification	**N**	**%**	**N**	**%**
Microfilaria, NOS, referred for identification	60	98.4	826	91.3
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	1	1.6	72	8.0

Discussion

Causal Agents

Perstans filariasis is caused by *Mansonella perstans*, a filarid nematode that occurs in Africa and South America.

Biology and Life Cycle

Adults of *M. perstans* reside in the peritoneal or pleural cavity (less commonly in the pericardium). Adults shed unsheathed microfilariae into the bloodstream where they circulate without specific periodicity. Microfilariae are ingested by an appropriate arthropod host (biting midges in the genus *Culicoides*). Microfilariae migrate from the mouthparts of the midge to the thoracic muscles via the hemocoel. There, the microfilariae develop into L1 larvae and eventually into L3 larvae. L3 larvae migrate to the mouthparts of the midge, where they infect the definitive host when the midge takes a blood meal.

Diagnosis

Diagnosis of *M. perstans* is made by the finding of characteristic unsheathed microfilariae on blood films. Microfilariae of *M. perstans* do not exhibit specific periodicity and may be found at all hours of the day or night. Microfilariae are small, measuring 190-200 µm long in blood films. The nuclear column is compact; nuclei extend to the end of the tail which is typically blunty-rounded. The following table will help differentiate *M. perstans* from the other two *Mansonella* spp. of humans, *M. ozzardi,* and *M. streptocerca*. In parts of the Democratic Republic of Congo (DRC), *M. perstans* should be differentiated from 'Microfilaria' *semiclarum*, an enigmatic species of uncertain

affinities. Microfilariae of 'M' *semiclarum* are larger (198-221 µm long, on average) and have a clear area measuring approximately 40 µm mid-body that is nearly devoid of nuclei, possessing only a few large, scattered nuclei.

Comparison of the human microfilariae found in blood specimens.

Species	Epidemiology	Measurements	Other Characteristics
Wuchereria bancrofti	Pantropical	244-296 µm long by 7.5-10.0 µm wide	Usually sheathed, sheath usually colorless with Giemsa, pH 7.0; tail tapered, anucleate; short head space
Brugia malayi	Southeast Asia to the Indian Subcontinent	177-230 µm long by 5-6 µm wide	Usually sheathed, sheath usually bright pink with Giemsa, pH 7.0; tail tapered with terminal and subterminal nuclei separated by gaps; long head space
Brugia timori	Lesser Sunda Archipelago (Timor, Sumba, Lembata, Pantar, Alor)	310 µm long by 6-7 µm wide	Usually sheathed, sheath usually colorless with Giemsa, pH 7.0; tail tapered with terminal and subterminal nuclei separated by gaps; long head space
Loa loa	West-central Africa	231-250 µm long	Usually sheathed, sheath usually colorless with Giemsa; tail nuclei irregularly spaces to the tip; short head space
Mansonella perstans	Sub-Saharan Africa, Central and South America, the Caribbean	199-200 µm long	Lacks sheath; tail bluntly-rounded with nuclei to the tip
Mansonella ozzardi	Central and South America, the Caribbean	163-203 µm long	Lacks sheath; tail tapered to a point and anucleate

Clinical Significance

Most people with *M. perstans* infection are asymptomatic. When symptoms are present they are usually related to migration of adult worms and include cutaneous swelling, angioedema, pruritis, fever, headaches, arthralgias and neurologic manifestations.

Treatment

Mansonella perstans infection is one of the more difficult human filarial infections to treat as it is relatively resistant to standard antifilarial agents. Regimens of albendazole or mebendazole are used but they do not clear microfilaremia in the majority of patients. The presence of an intracellular endosymbiont called *Wolbachia* within *M. perstans* in Mali and Cameroon has been demonstrated, leading to successful treatment of *M. perstans* with doxycycline. However, this treatment has not been evaluated in other parts of the world. Treatments for the other species of *Mansonella* are more effective. The drug of choice for *M. ozzardi* is ivermectin and for *M. streptocerca* it is diethylcarbamazine.

References

1. Simonsen PE, Onapa AW, Asio SM. *Mansonella perstans* filariasis in Africa. *Acta Trop.* 2011 Sep;120 Suppl 1:S109-120.
2. Coulibaly YI, Dembele B, Diallo AA, et al. A randomized trial of doxycycline for *Mansonella perstans* infection. *N Engl J Med*. 2009 Oct;361(15):1448-1458

Blood Parasite Identification

The BP-12 challenge contained thick and thin Giemsa-stained smears obtained from a 55-year-old female returning from a rain forest expedition in West Africa. The specimen contained *Loa loa*. A response of "Microfilaria-*Loa loa*", "Microfilaria, NOS, referred for identification", or "Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification" would have been considered satisfactory; however, consensus was not reached for parasite identification or parasite screen.

Table 1. Parasite Identification

Total Responses	Referees (55)		Participants (424)	
Identification	**N**	**%**	**N**	**%**
Microfilaria-Loa loa	27	49.1	180	42.5
Unacceptable				
Microfilaria-*Mansonella* sp.	6	10.9	36	8.5
Plasmodium sp., NOS, would refer or request another specimen, or perform additional molecular testing	1	1.8	4	0.9

Table 2. Parasite Screen

Total Responses	Referees (62)		Participants (895)	
Identification	**N**	**%**	**N**	**%**
Microfilaria, NOS, referred for identification	26	41.9	337	37.6
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	7	11.3	24	2.7

Discussion

Causal Agents

Loaisis is caused by the 'African eye worm', *Loa loa*. This filarid nematode is distributed in equatorial rain forest areas in West and Central Africa, south of the Sahara.

Biology and Life Cycle

Loa loa is transmitted by deer flies in the genus *Chrysops*. While taking a blood meal, infected deer flies introduce infective L3 larvae onto the skin of the human host, where they penetrate the bite wound. The L3 larvae develop into adults that usually reside in subcutaneous tissues, although adults can be recovered from the eye. Adults mate and females release microfilariae into the blood where they circulate during the day (diurnal periodicity). Deer flies become infected when they take a blood meal during the day while microfilariae are circulating. After ingestion, microfilariae shed their sheaths and migrate from the fly's midgut through the hemocoel to the thoracic muscles. There, the microfilariae develop into L1 and eventually L3 larvae. L3 larvae migrate to the fly's proboscis and infect another human when the fly takes a blood meal.

Diagnosis

Loiasis is typically diagnosed by the finding of microfilariae in peripheral blood films. *Loa loa* exhibits a diurnal periodicity and the optimal time to collect blood is between 10AM and 2PM. Concentration techniques (such as

Knott's technique) can enhance diagnosis. Microfilariae may also be found in urine, sputum, and CSF. Microfilariae are 231-250 µm long and possess a sheath that does not stain with Giemsa. Sheaths are not always evident in stained smears. The tail of the microfilaria is tapered, with nuclei extending to the tip of the tail. Adults may be recovered from the eye. Adult females measure 50-70 mm long, while the males are smaller at 30-35 mm long. Adult worms have characteristic bosses on the cuticle surface. There are no routine serologic or molecular tests available for loaisis.

Clinical Significance

Infection with the *L. loa* parasite is usually asymptomatic in patients and often seen in returning travelers and immigrants from West and Central Africa. The bite of the *Chrysops* vector itself can be painful and cause redness, swelling and itchiness. However, symptoms can develop after several weeks including angioedema localized typically to the upper limbs but sometimes the lower limbs and facial area. These so called "Calabar swellings" are often red and itchy in nature and thought to be an inflammatory response to the worm or its metabolic product lasting up to three days. Migration of the adult worm is not usually symptomatic, and classically noted on the upper nose or in the conjunctiva of the eye (more often seen in patients from endemic areas rather than travelers). After several months, adult worm egg packets or microfilaria can be found in the blood, lungs, spinal fluid and sputum of most infected individuals. Eosinophilia is observed in peripheral blood. *Loa loa* has also been known to cause hydroceles and orchitis in males, as well as bowel, kidney and central nervous system lesions.

Treatment

The mainstay of treatment involves pharamacotherapy. Both diethylcarbamazine (DEC) and ivermectin have been used for this purpose. Diethylcarbamazine is first line and given over a period of 21 days; it is effective primarily against the microfilaria and to a lesser degree the adult worms. The action of DEC against a high microfilaria burden has been linked to encephalopathy and death as a severe complication. Concomitant anti-inflammatory medication can reduce the risk of side effects. Surgical incision and removal of adult worms may also be indicated, for example the subconjunctival space under local anesthesia.

References

1. Ash LP, Orihel TC. *Atlas of Human Parasitology*, 5th ed. Chicago, IL: ASCP Press: 2007.
2. Centers for Disease Control and Prevention, Division of Parasitic Diseases (DPD). *Laboratory Identification of Parasites of Public Concern.* http://www.cdc.gov/dpdx
3. Mandell GL, Bennett JE, Dolin R. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 7th ed. Philadelphia, PA: Churchill Livingstone Elsevier, 2009.

Blood Parasite Identification

The BP-13 challenge contained thick and thin Giemsa-stained smears obtained from a 50-year-old man with no documented international travel. A response of "No parasite(s) seen" or "Specimen screened for blood parasites, no organisms seen" was considered satisfactory.

Table 1. Parasite Identification

Total Responses	Referees (59)		Participants (471)	
Identification	**N**	**%**	**N**	**%**
No parasite(s) seen	59	100.0	465	98.7

Table 2. Parasite Screen

Total Responses	Referees (58)		Participants (857)	
Identification	**N**	**%**	**N**	**%**
Specimen screened for blood parasites, no organisms seen	58	100.0	834	97.3

Discussion

Identification

Careful examination of multiple thin and thick blood films is imperative to exclude the diagnosis of blood parasites, particularly for patients living in endemic areas. For thin films: (1) all blood components (erythrocytes, white blood cells, and platelets) should be intact, (2) the background should be clean and free from debris, (3) erythrocytes should stain a pale grayish-pink, and neutrophilic leukocytes should have deep purple nuclei and well defined granules, and (4) erythrocytes at the terminal, feathered end of the film should be adjacent, but not overlap (one layer thick). For thick films: (1) the background should be clean, free from debris, with a pale mottled-gray color derived from lysed erythrocytes, (2) leukocytes should stain deep purple with pale purple cytoplasm, and (3) eosinophilic granules should stain a bright purple-red and neutrophilic granules should stain deep pink-purple.

Thick films are most useful for screening since they provide a larger quantity of blood for examination. Thin films, on the other hand, are most useful for speciation since they provide the best red blood cell (RBC) and parasite morphology. All requests for peripheral blood smear examination to detect *Plasmodium* spp. should be performed without delay. Both thick and thin films should first be fully screened at low power (ie, using the 15x objective) to detect microfilaria which may be present in low numbers anywhere on the slides and which may not be detected in the standard 300 field slide review at higher magnification.

Due to the severe implications of a misdiagnosis, laboratory personnel should then examine at least 300 oil immersion fields (using the 150X oil immersion objective) for each thick and thin blood film. In addition, one set of blood films is not sufficient to exclude the diagnosis of malaria and the laboratory should recommend collection of multiple blood specimens approximately at 6-8 hour intervals to definitively exclude the presence of blood parasitemia. This comment should accompany the final report "No blood parasites seen."

References

1. Garcia LS. 2016. *Diagnostic Medical Parasitology*, 6th ed., Washington, DC. ASM Press.

Blood Parasite Identification

The BP-14 challenge contained thick and thin Giemsa-stained smears obtained from a 60-year-old male undergoing organ transplantation who emigrated from Mexico 30 years prior. The specimen contained *Trypanosoma cruzi*. A response of "*Trypanosoma cruzi*", "Blood flagellate, NOS, referred for identification", or "Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification" were considered satisfactory.

Table 1. Parasite Identification

Total Responses	Referees (61)		Participants (484)	
Identification	**N**	**%**	**N**	**%**
Trypanosoma cruzi	61	100.0	482	99.6

Table 2. Parasite Screen

Total Responses	Referees (56)		Participants (839)	
Identification	**N**	**%**	**N**	**%**
Blood flagellate, NOS, referred for identification	49	87.5	733	87.4
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	4	7.1	87	10.4

Discussion

Causal Agent

American Trypanosomiasis (also called Chagas disease) is caused by *Trypanosoma cruzi*, a flagellated protozoan endemic to the American tropics. Although *T. cruzi* is enzootic in the United States, the feeding/defecation patterns of the Nearctic triatomine bugs, in combination with improved living conditions, do not allow for efficient vector-borne transmission.

Biology and Life Cycle

Trypanosoma cruzi is transmitted by triatomine ('kissing') bugs as the bug releases infective trypomastigotes in the feces while taking a blood meal. Trypomastigotes enter the bite site when scratched into the wound, or other mucus membranes such as the conjunctiva. *Trypanosoma cruzi* has also been transmitted in fruit juices and other foods, when infected bugs contaminate fruits and other food sources. At the infection site, parasites differentiate into intracellular amastigotes. Amastigotes multiply by binary fission and differentiate into trypomastigotes and are released into the bloodstream. Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in the new infection sites. Only amastigotes replicate; trypomastigotes do not divide (unlike with the African trypanosome, *T. brucei*). Triatomine bugs become infected when they take a blood meal from an infected human or animal with circulating trypomastigotes. Ingested trypomastigotes transform into epimastigotes in the midgut and multiply there. Epimastigotes migrate to the hindgut where they become infective metacyclic trypomastigotes.

Diagnosis

Trypanosoma cruzi can be challenging to diagnose. During the acute stage of the disease, trypomastigotes may be observed in peripheral blood or CSF. Trypomastigotes are approximately 20 µm long, have a central nucleus, and a large subterminal kinetoplast at the pointed posterior end. The single flagellum is anteriorly directed. Dividing forms are not seen.

During the chronic stage of the disease, amastigotes may be found in tissue biopsy specimens, although serologic testing is recommended. Molecular diagnosis (PCR) is often employed in cases of transplant or transfusion transmission or when congenital cases are suspected. PCR can also be useful for early detection of *T. cruzi* in transplant-transmitted recipients of organs from donors with chronic disease. The diagnosis of chronic Chagas in patients without immunosuppression should be performed with serology.

Clinical Significance

Between six and seven million people are thought to be infected with *T. cruzi* in the Americas. The clinical presentation of Chagas is biphasic. Acutely, over a period of two months, individuals can be asymptomatic or present with skin changes such as swelling of eyelids accompanied with fever, myalgia, and lymphadenopathy. Disease with this pathogen can be cured if treated early. Complications of chronic disease include heart (30%), gastrointestinal (10%), neurological (5%), and mixed disease. If untreated, cardiomyopathy and neurological deficits can lead to sudden death. Blood donor and organ screening is critical to prevent transfusion or organ related transmission. Other forms of transmission include consumption of food contaminated with triatomine excrement, congenital infection, and laboratory accidents usually with infected human specimens.

Treatment

Specific anti-Chagas drug therapy can be achieved with benznidazole and nifurtimox. Both agents are effective in the acute phase, but efficacy is proportionally lower as the disease progresses into the chronic phase. Treatment in the acute phase can be protracted (up to 2 months) and complicated by adverse drug reactions such as kidney and liver injury. Cardiac and gastrointestinal disease may require targeted therapy to correct the anatomical dysfunction caused by chronic disease. Immunosuppressive regimens associated with autoimmune or neoplastic disease can lead to reactivation of Chagas which also requires anti-parasitic therapy.

References

1. Ash LP, Orihel TC. *Atlas of Human Parasitology*, 5th ed. ASCP Press: 2007.
2. Centers for Disease Control and Prevention, Division of Parasitic Diseases (DPD). *Laboratory Identification of Parasites of Public Concern.* Accessed February 24, 2021. http://www.cdc.gov/dpdx
3. Mandell GL, Bennett JE, Dolin R. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 7th ed. Churchill Livingstone Elsevier, 2009.

Blood Parasite Identification

The BP-15 challenge contained thick and thin Giemsa-stained smears obtained from a 65-year-old male who had emigrated 15 years ago from Malaysia. The specimen contained *Plasmodium malariae* and *Plasmodium falciparum*. A response of "*Plasmodium malariae*", "*Plasmodium malariae/P. knowlesi*", "*Plasmodium* sp., not P. *falciparum*, referred for identification", "*Plasmodium* sp., NOS, would refer or request another specimen, or perform additional molecular testing", and "*Plasmodium falciparum*", or "*Plasmodium* sp./*Babesia* sp. seen, referred for identification" was considered satisfactory. For consensus to be reached, correct responses for both organisms must be reported. However, consensus was not reached for parasite identification of both organisms.

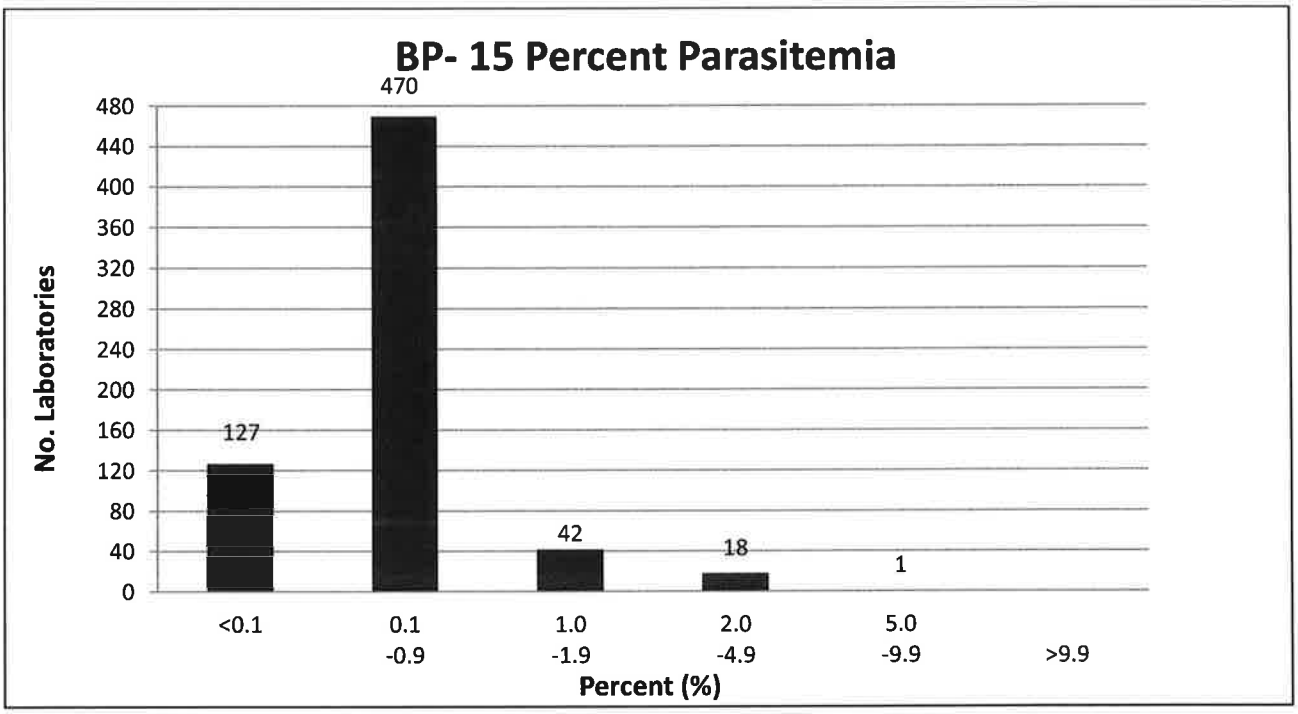
Table 1. Parasite Identification

Total Responses	Referees (62)		Participants (512)	
Identification	**N**	**%**	**N**	**%**
1st Organism				
Plasmodium malariae	14	22.6	94	18.3
Plasmodium malariae/P. knowlesi	6	9.7	65	12.7
Plasmodium sp., not *P. falciparum,* referred for identification	16	25.8	94	18.3
Plasmodium sp., NOS, would refer or request another specimen, or perform additional molecular testing	12	19.4	95	18.4
2nd Organism				
Plasmodium falciparum	22	35.5	244	47.7
Consensus for correct identification of both organisms	22	35.5	177	34.6
Unacceptable				
Plasmodium vivax/ovale, NOS	4	6.5	10	1.9
Babesia sp.	1	1.6	1	0.2

Table 2. Parasite Screen

Total Responses	Referees (55)		Participants (812)	
Identification	**N**	**%**	**N**	**%**
Plasmodium sp./*Babesia* sp. seen, referred for identification	53	96.4	800	98.5

If you have identified a *Plasmodium* sp. or *Babesia* sp.: Report percent of infected RBCs seen (number of infected RBCs per 100 red blood cells). (Ungraded)



Discussion

Identification

Both thick and thin blood films should be prepared when the diagnosis of malaria is suspected. Examination of the thick blood film is considered the gold standard for diagnosis because a larger blood volume can be examined enabling the detection of low levels of parasitemia. Thin blood films are helpful with species identification. In this Survey, the primary challenge was speciation of *Plasmodium malariae* and *Plasmodium faciparum*.

Key morphologic features on thin and thick blood film that suggested the diagnosis of *P. malariae* are:

1. Normal size and shape of infected erythrocytes.
2. Predilection to infect older red blood cells.
3. Growing and mature trophozoites, with few to no ring forms. The "band-shaped form" is characteristic and highly suggestive of the diagnosis.
4. Absence of Schuffner's dots and normal color cytoplasm.
5. Schizonts with 6-12 merozoites with rosette pattern.
6. Rounded and compact gametocytes.

Key morphologic features on thin blood film that suggests a diagnosis of *P. falciparum* may include:

1. Normal size and shape of infected erythrocytes.
2. Smaller, more delicate ring-form trophozoites (approximately 1/5 the diameter of the erythrocyte) frequently with two chromatin dots (so-called "head phone" forms).
3. Erythrocytes infected with multiple parasites.
4. Presence of ring-form trophozoites at the edge of the erythrocyte (appliqué forms).
5. Usually an absence of mature trophozoites and schizonts in the peripheral blood film (may be seen if there is a delay in processing the blood specimen, or in patients with a very high parasitemia or underlying conditions).
6. Presence of crescent-shaped gametocytes (not always seen).
7. Absence of Schüffner's stippling. Larger, comma-shaped dots (Maurer's clefts) may be seen, especially when the stain buffer is at a pH of 7.2.

Causal Agents

There are four species of *Plasmodium* that cause human malaria: *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*. *Plasmodium ovale* is sometimes divided into two subspecies, which may actually represent valid species, *P. o. walkeri* and *P. o. curtisi*. In addition, there are at least six species of simian *Plasmodium* that have been implicated in causing zoonotic infections in humans, the most notable being *P. knowlesi*, human infections of which appear to be increasing on the Malaysian peninsula (see also Table 3).

Plasmodium falciparum occurs nearly worldwide in the tropics and subtropics, particularly in Africa and southeast Asia. *Plasmodium malariae* also occurs nearly worldwide in the tropics and subtropics but has a patchier distribution than *P. falciparum*; most common in tropical Africa, Indian subcontinent, and southeast Asia. *Plasmodium ovale* occurs primarily in tropical western Africa, but also New Guinea and southeast Asia; *P. ovale* has not yet been documented from the New World. *Plasmodium vivax* occurs nearly worldwide in the tropics, subtropics, and some regions of northern and eastern Africa, the Central Asia, the Indian subcontinent, southeast Asia, and the Americas.

Table 3.
Plasmodium species recorded infecting humans, their geographic distributions, and natural intermediate (mammalian) hosts.

Species	Geographic Distribution	Natural Intermediate Hosts
*Plasmodium brasilianum**	South America	Howler monkeys, spider monkeys, tits, capuchins, bearded saki, woolly monkeys, squirrel monkeys
Plasmodium cynomolgi	Southeast Asia	Macaques, surilis
Plasmodium falciparum	Circumtropical	Humans
Plasmodium inui	Southeast Asia	Macaques
Plasmodium knowlesi	Southeast Asia	Macaques
Plasmodium malariae	Africa (primarily tropical sub-Saharan), southeast Asia, South America, southern Central America, Caribbean	Humans
Plasmodium ovale	Africa (primarily western and tropical sub-Saharan), southern and southeastern Asia	Humans
Plasmodium schwetzi	Tropical western Africa	Chimpanzees, gorillas
*Plasmodium simium**	Brazil	Howler monkeys
Plasmodium vivax	Africa (East, Horn of Africa and Madagascar), Central and South America, Central Asia, Indian Subcontinent, Southeast Asia, Korean Peninsula	Humans

*Molecular data suggest *P. brasilianum* and *P. simium* may actually just be *P. malariae* and *P. vivax*, respectively, which adapted to non-human primates after introduction to South America.

Biology and Life Cycle

Plasmodium spp. are transmitted by mosquitoes in the genus *Anopheles*. Infected female mosquitoes inject sporozoites when taking a blood meal. Sporozoites are carried via blood to the liver where they invade hepatocytes and form schizonts. The liver schizonts rupture, releasing large numbers of merozoites that then invade erythrocytes starting the erythrocytic cycle. With *P. vivax* and *P. ovale*, some parasites will remain in the liver as hypnozoites, resulting in relapses months or years later. Early ring forms develop into mature trophozoites and take one of two pathways: 1) they develop into schizonts (which rupture and continue the erythrocytic cycle) or 2) develop into gametocytes. Gametocytes are a dead-end stage in the human host but are required for sexual reproduction in the mosquito. In the mosquito host, microgametocytes (=males) exflagellate and fertilize macrogametocytes (=females), resulting in an ookinete. Ookinetes further develop into oocysts, which when mature rupture and release the infective sporozoites. In *P. falciparum*, late trophozoites and schizonts express a protein on the surface of the erythrocytic membrane causing the infected erythrocyte to adhere to the endothelial lining of capillaries in internal organs. Thus, only ring forms and gametocytes are usually seen in well-prepared peripheral blood smears.

Diagnosis

General Considerations

Malaria is primarily diagnosed by the identification of *Plasmodium* parasites on thin and thick blood films stained with Giemsa, Wright, or Wright-Giemsa stain. Molecular methods such as PCR may be employed when an identification cannot be made morphologically or there is morphologic evidence of a mixed infection. Serology is not used for routine diagnosis but may be helpful during transfusion investigations.

Typically, thick films are used for the recognition of *Plasmodium*, with a species-level identification performed on the thin film. Thin films should be read at 1000x magnification with oil for at least 100 microscopic fields. Immunologically naïve patients (e.g. returning travelers born in non-endemic areas) may present with stronger clinical manifestations at a lower parasitemia. Sever malaria is currently defined as a parasitemia $\geq$ 2% in immunologically naïve patients and $\geq$ 5% in non-naïve patients.

Morphologic Identification

Two important questions regarding morphologic identification of malaria are:

1) is it malaria?
2) is it *Plasmodium falciparum*?

Recognition of *Plasmodium* is based on observing stages of the parasite inside infected red blood cells. In a well-prepared specimen, the cytoplasm will stain blue and the chromatin red. Pigment (which is absent in *Babesia* infections) will present as golden-brown to black flecks. In some species, structures such as Schüffner's stippling or Maurer's clefts may be present when stained at an appropriate pH. Identification of *Plasmodium* to the species level is extremely important for patient management, as different species may be treated differently (for example, it is important to target the liver stages of *P. ovale* and *P. vivax* to prevent relapse of the disease).

Table 4.
The following table compares the morphologic features of the four stages of human *Plasmodium* spp.

Morphologic Criteria	*Plasmodium falciparum*	*Plasmodium malariae*	*Plasmodium ovale*	*Plasmodium vivax*
Size of infected RBC	Normal	Normal to smaller	Enlarged	Enlarged
Rings (early trophozoites)	Common; usually with thin, delicate cytoplasm and double chromatin dots; often multiple rings per infected RBC; appliqué forms common	Cytoplasm sturdy, usually with single, large chromatin dot; occasional 'birds-eye' forms	Cytoplasm sturdy, with 1-2 large chromatin dots	Large, sturdy cytoplasm, usually with large, single chromatin dot
Developing trophozoites	Rare, but may be seen if a delay in processing; form compact, pigment usually evident	Variable; may be compact to elongate (band-form) or pleomorphic and vacuolated (basket-form); pigment coarse	Compact to slightly amoeboid with dark pigment; elongation and fimbriation may be observed	Pleomorphic to grossly amoeboid; pigment diffuse and golden-brown to nearly black
Schizonts	Rare in peripheral blood; 8-24 small merozoites when mature; pigment dark, compact	6-12 merozoites when mature, often in a rosette pattern around central mass of pigment	6-14 merozoites when mature; pigment dark brown to black and discrete when mature; elongation and fimbriation may be present	12-24 merozoites when mature; may fill entire RBC; often noticeable enlarged
Gametocytes	Crescent-shaped; chromatin discrete (macrogametocyte) or diffuse (microgametocyte); Laveran's bib may be present	Small, round, compact; pigment coarse and diffuse	Round to oval, compact; if elongated and fimbriated may not fill entire infected RBC; pigment coarse, dark	Large and round to pleomorphic (may 'hug' surrounding RBCs); may fill most of infected RBC; pigment golden-brown to nearly black
Other Features	Maurer's clefts may be present; ring-form trophozoites usually predominate	Generally smaller; pigment coarse; Ziemann's stippling may be present; all stages seen	Schüffner's stippling may be present at appropriate pH; elongation and fimbriation may be observed; all stages seen	Schüffner's stippling may be present at appropriate pH; enlargement of infected RBCs usually pronounced; all stages seen

Calculating Percent Parasitemia

The percent parasitemia is very important to calculate for prognostic purposes and also to evaluate response to antimalarial therapy.

Parasitemia can be calculated on a thin blood film as follows:

1. Count the number of infected RBCs in relation to uninfected RBCs. Between 1,000-10,000 RBCs should be counted (ideally at least 1000).
2. Apply the formula:

$$\frac{\text{\# of parasitized RBCs}}{1000 \text{ (or } 200)} \times 100 = \% \text{ parasitemia}$$

Procedural notes:

- an infected RBC containing multiple parasites is calculated only once
- fields devoid of parasites should be included, if encountered
- gametocytes should not be included in the count. The justification is because 1) some antimalarial drugs are not gametocidal and the presence of gametocytes post-treatment is not indicative of the effectiveness of the treatment and 2) gametocytes are a dead-end stage in the human host.

Important parasitemia thresholds

Clinicians take several components into consideration, including the percent parasitemia, when making treatment decisions. In general, levels of parasitemia ≥ 5% are indicative of severe disease and should be treated aggressively with parenteral antimalarial therapy in all patients (see also Treatment, below). A lower threshold parasitemia of ≥ 2% may indicate severity in the non-immune traveler. Other clinical criteria that are indicative of severe disease include impaired consciousness, renal failure, severe anemia (Hb <7g/dL), acute respiratory distress syndrome, hemoglobinuria, jaundice, hypotension, disseminated intravascular coagulation, and spontaneous bleeding. In patients with *P. falciparum* or *P. knowlesi,* hyperparasitemia (parasitemia >10%), more aggressive interventions may be considered. The role of exchange transfusion is controversial but may be useful for removing parasites from the blood stream, improving oxygen carrying capacity and improving blood viscosity.

Calculating parasites/microliter on a thick film

In endemic areas where thin films are not prepared, the parasite load (parasites/microliter) can be determined using the thick film. It is helpful to know the patient's WBC count prior to performing the calculations. While reading a thick smear, count the number of parasites and WBCs. Stop counting when one of the two scenarios have been accomplished: 1) ≥ 100 parasites **and** 200 WBCs have been counted, or 2) ≤ 99 parasites **and** 500 WBCs have been counted. If the patient's WBC count is unknown, use the assumption of 8,000 WBCs/μL of blood for the final calculation (below).

The final calculation can be made as follows:

$$\text{Parasites/}\mu\text{L blood} = \frac{\text{No. parasites counted} \times 8000 \text{ WBCs/}\mu\text{L (or patient's known count)}}{\text{No. WBCs counted}}$$

Molecular Detection

Molecular detection and identification of *Plasmodium* species is becoming increasingly popular in the diagnostic laboratories, although it can often be cost prohibitive for some labs, especially smaller labs with a lower specimen volume. There are currently no FDA-approved commercial assays for routine clinical use in the United States, and to date all available assays are laboratory-derived tests (LDTs). However, several molecular assays are approved for use in Europe and Canada. Multiple assays have been described, including DNA/RNA hybridization, loop-mediated isothermal amplification (LAMP), conventional and real-time PCR, and nucleic acid sequence-based amplification (NASMA). The preferred specimen type for molecular detection is whole blood collected in EDTA,

although several assays have been validated for finger-stick blood collected on dried blood spots such as filter papers.

Table 5.
The following table highlights the advantages and disadvantages for the molecular detection of *Plasmodium* species (adapted from Mathison and Pritt 2017)

Advantages	Disadvantages
• More sensitive than microscopy and RDTs • Less subjective than microscopy • Improved diagnosis of mixed infections • Requires less training time of personnel than microscopy • Allows for detection of polymorphisms associated with drug resistance.	• Still cost-prohibitive in many places, especially for routine diagnosis • Often not performed on a STAT basis • High-complexity method that requires special training of personnel • Should not be used to evaluate treatment success

Antigen Detection
There are over 40 rapid detection tests (RDTs) commercially available on a worldwide basis for the detection of *Plasmodium*. However, in the United States, there is only one that is approved by the FDA for human use, the BinaxNOW® Malaria Test (Abbott, Chicago, IL). This test targets *P. falciparum*-specific Hrp2 and aldolase common to the four human species of *Plasmodium*. According to the package insert, the BinaxNOW® test has sensitivities for the detection of *P. falciparum* and P. vivax of 100% and 81.6%, respectively, using blood obtained by venous draw, however the sensitivity drops to 30% for other species. Regardless of the results when performing the BinaxNOW® Malaria Test, the results should be confirmed my microscopy. Also, the BinaxNOW® Malaria Test should not be used to monitor treatment success as residual antigen can result in false-positive results for as long as 28 days in the case of Hrp2.

Antibody Detection
Antibody detection is not typically recommended for routine clinical diagnosis of malaria, except for a few clinical scenarios, including but not limited to: 1) febrile patients with recent travel to endemic areas that are repeatedly smear negative, 2) diagnosis of suspected tropical splenomegaly syndrome, and 3) trace-back investigations of donors in transfusion-associated cases.

Clinical Significance
Nearly half of the world's population is at risk of malaria. In 2019, there were an estimated 229 million cases of malaria worldwide, with 409,000 deaths. Most malaria cases (94%) and deaths (94%) occur sub-Saharan Africa. There are approximately 1,000 cases of malaria diagnosed in the United States each year, almost all in recent travelers and immigrants. The visiting friends and relatives (VFR) population contribute to the vast majority of cases in travelers returning to non-endemic areas. Although the *Anopheles* mosquito is endemic in parts of North America, malaria transmission was largely eliminated in the 1940s through public health efforts.

Malaria infection can be classified as either uncomplicated or severe (complicated). In uncomplicated infections, patients present with nonspecific symptoms including fever, chills, sweats, headaches, nausea/vomiting, body aches and malaise. Symptoms classically (but infrequently observed) recur either in a two-day cycle (*P. falciparum*, *P. vivax* and *P. ovale*) or in three-day cycle (*P. malariae*). In severe infections, organ failure and/or metabolic abnormalities occur including severe anemia, acute respiratory distress syndrome, acute kidney failure, metabolic acidosis, cerebral infection, and coagulation abnormalities. Severe infection is considered a medical emergency requiring urgent treatment. *P. falciparum* and *P. knowlesi* can cause severe illness and death whereas *P. vivax*, *P. malariae* and *P. ovale* tend to cause less severe illness. The hypnozoite form of *P. vivax* and *P. ovale* can remain dormant in a patient's liver and cause relapsing infection.

Treatment

Treatment of malaria should ideally wait until a laboratory diagnosis has been made. Treating "presumptively" should occur only when no other option exists. Therapy is guided by the infecting species of *Plasmodium*, the clinical status of the patient and the drug susceptibility of the infecting parasites (dependent on geographic area and previous anti-malarial treatment). Because of the rapid progression of *P. falciparum* infections and a high risk of fatality, urgent treatment is essential.

If the infection is uncomplicated, oral anti-malarial medication can provide effective treatment. However, severe infections necessitate parenteral therapy. *Plasmodium falciparum* and *P. vivax* have different drug resistance patterns in different geographic regions. Although not readily available in North America, the WHO recommends artemisinin-based combination therapy as first-line treatment in uncomplicated *P. falciparum* malaria (oral administration), severe malarial infections (intravenous administration) and *P. vivax* infections in areas of known chloroquine resistance. Other, non-artemisinin based combination treatments include sulfadoxine-pyrimethamine plus either chloroquine or amodiaquine or atovaquone-proguanil. In recent years, resistance to artemisinins has been detected in Cambodia, Laos, Myanmar, Thailand and Viet Nam[3]. Chemoprophylaxis can be achieved with atovaquone-proguanil, doxycycline, and mefloquine as examples. For confirmed *P. vivax* and *P. ovale* infections, radical cure can be achieved with treatment using primaquine or tafenoquine, in order to prevent relapse due to the hypnozoite form, except in high-transmission settings where re-infection is likely. Mixed-species malarial infections are not common but may be underestimated by routine microscopy.

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