Blood Parasite Identification

BP-06 Introduction

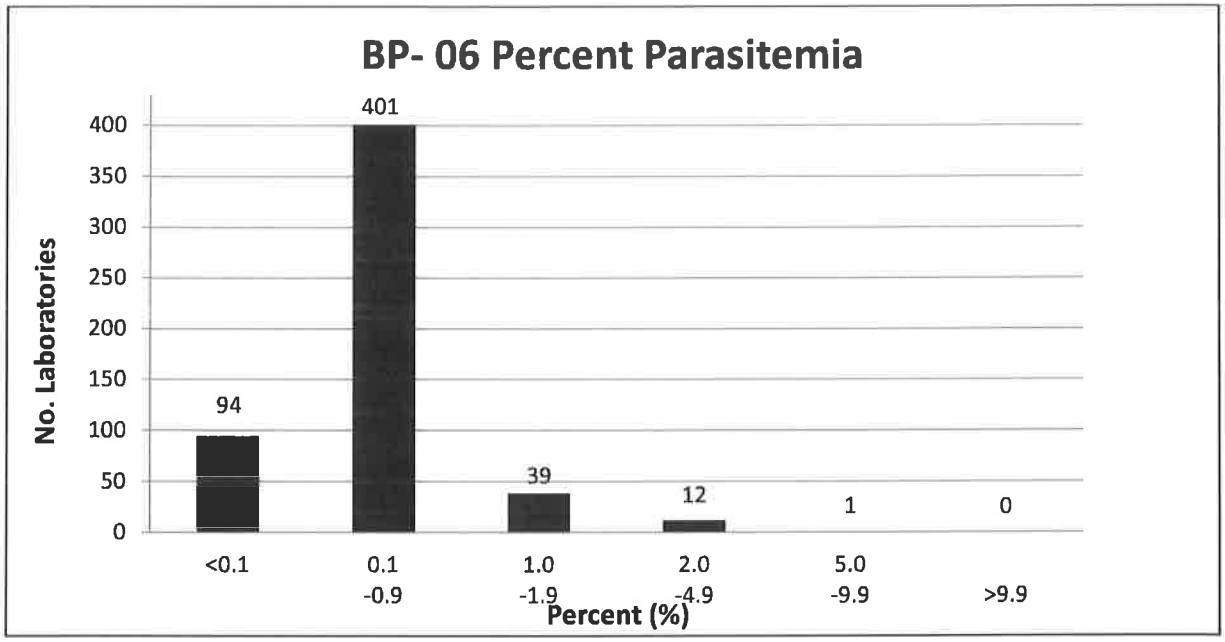
Thick and thin Giemsa-stained smears were obtained from a 49-year-old primatologist returning from field work in Zimbabwe. The specimen contained *Plasmodium ovale*. A response of "*Plasmodium ovale*", "*Plasmodium vivax/ovale* NOS", "*Plasmodium* sp., not *P. falciparum* referred for identification", "*Plasmodium* sp., NOS would refer or request another specimen, or perform additional molecular testing", or "*Plasmodium* sp./*Babesia* sp. seen referred for identification" was considered satisfactory.

BP-06

Parasite Identification	Referees (54) No.	%	Participants (375) No.	%
Plasmodium ovale	10	18.5	77	20.5
Plasmodium vivax/ovale, NOS	11	20.4	64	17.1
Plasmodium sp., not *P. falciparum*, referred for identification	16	29.6	115	30.7
Plasmodium sp., NOS, would refer or request another specimen, or perform additional molecular testing	6	11.1	49	13.1
Plasmodium malariae	8	14.8	52	13.9
Plasmodium vivax	1	1.9	12	3.2
Plasmodium falciparum	2	3.7	10	2.7

Parasite Screen	Referees (58) No.	%	Participants (755) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	57	98.3	751	99.5

BP-06	**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)**



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 identification since a larger blood volume can be examined than with the thin film, thus enabling the detection of low levels of parasitemia. In contrast, thin blood films provide the best morphology for species differentiation. In this Survey, the challenge was to recognize the presence of *Plasmodium ovale.*

Key morphologic features on thin and thick blood films that characterize *P. ovale* may include:

1. Enlarged size of the infected red blood cells (seen with both *P. ovale* and *P. vivax).*
2. Infected red blood cells with fimbriations and an ovoid shape (seen in up to 1/3 of infected cells)
3. Presence of Schüffner's stippling (seen in both *P. ovale* and *P. vivax*). Note: These may not be seen in early ring-form trophozoites in *P. ovale*
4. Mature schizonts with 8-12 merozoites
5. Compact ring compared to the more amoeboid trophozoite of *P. vivax.*
6. Large, round gametocytes usually with coarser pigment than as usually seen with *P. vivax.*

Distinguishing *P. ovale* from *P. vivax* morphologically may be challenging when the trophozoites do not have characteristic features and fimbriations are not abundant. The absence of schizonts also makes the differentiation between the two species more difficult. In these cases a diagnosis of *Plasmodium vivax/ovale* may be acceptable. If available, molecular testing can be used confirm the species when morphology is not definitive.

Note: *The ideal specimen for laboratory identification of malaria is fresh capillary blood from a finger or heel stick, with immediate preparation of thick and thin blood films. Since this is not feasible in most settings, however, venous blood collected in EDTA anticoagulant is also acceptable. It is important to transport the blood as quickly as possible to the laboratory for examination since prolonged exposure to EDTA may result in distortion of the malaria parasites and compromised morphology.*

Discussion

Causal Agents

There are four species of *Plasmodium* that cause human malaria: *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*. In addition, there are at least six species of simian *Plasmodium* that have been documented to cause zoonotic infections in humans, the most notable being *P. knowlesi*, infections of which appear to be increasing on the Malaysian peninsula.

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 more patchy 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 Middle East, the Indian subcontinent, Southeast Asia, and the Americas.

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. 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

The ideal specimen for laboratory identification of malaria is fresh capillary blood from a finger or heel stick, with immediate preparation of thick and thin blood films. Since this is not feasible in most settings, however, venous blood collected in EDTA anticoagulant is also acceptable. It is important to transport the blood as quickly as possible to the laboratory for examination since prolonged exposure to EDTA may result in distortion of the malaria parasites and compromised morphology.

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. Rapid diagnostic tests are also commonly used to distinguish *P. falciparum* from other malaria infections. 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 (eg, returning travelers born in non-endemic areas) may present with stronger clinical manifestations at a lower parasitemia. Severe malaria is currently defined as a parasitemia ≥ 2% in immunologically naïve patients and ≥ 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).

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 per 100 RBCs in different oil immersion fields.
2. Apply the formula:

$$\frac{\text{\# of infected RBCs}}{\text{total \# of RBCs counted}} \times 100 = \% \text{ parasitemia}$$

Notes:

1) At least 500 RBC's should be counted, with counting 2000 or more RBCs providing the most accurate estimation of parasitemia
2) An infected RBC containing multiple parasites is calculated only once
3) Fields devoid of parasites should be included, if encountered
4) Gametocytes should not be included in the count. Justification is because: a) many antimalarial drugs are not gametocidal and the presence of gametocytes post-treatment is not indicative of the effectiveness of the treatment and b) gametocytes are a dead-end stage in the human host.

Clinical Significance

In 2014, ninety-seven countries and territories had ongoing malaria transmission. Over half a million people die from malaria each year. Most malaria cases and deaths occur in children in sub-Saharan Africa. In 2011, a 40-year high of 1,925 cases of malaria were reported to the CDC in the United States, almost all in recent travelers and immigrants. Although the *Anopheles* mosquito is endemic in parts of North America, malaria transmission was largely eradicated 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. *Plasmodium falciparum* 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, amodiaquine, or atovaquone-proguanil. In recent years, resistance to artemisinins has been detected in Cambodia, Laos, Myanmar, Thailand and Vietnam. For confirmed *P. vivax* and *P. ovale* infections, radical cure can be achieved with treatment using primaquine and in order to prevent relapse due to the hypnozoite form. In high-transmission settings re-infection with *P. vivax* is likely. Mixed-species malarial infections are not common but may be underestimated by routine microscopy.

References

1. Centers for Disease Control and Prevention. Treatment of Malaria: Guideline for Clinicians. Accessed June 16, 2020. http://www.cdc.gov/malaria/diagnosis_treatment/clinicians3.html
2. World Health Organization. Guidelines for the Treatment of Malaria. 3rd ed. Geneva, 2015.
3. World Health Organization. Malaria: Fact Sheet #94. Updated December 2014. Accessed June 16, 2020. http://www.who.int/mediacentre/factsheets/fs094/en/
4. Garcia LS. *Diagnostic Medical Parasitology*. 5th ed. ASM Press; 2007.
5. CDC: Malaria surveillance – United States 2005. *MMWR* 2007;56(SS06);23-38.
6. Greenwood BM, Bojang K, Whitty CJ, Targett GA. Malaria. *Lancet*. 2005;365:1487-1498

BP-07 Introduction

Thick and thin Giemsa-stained smears were obtained from a 29-year-old female residing in Paraguay. 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.

BP-07

Parasite Identification	Referees (54) No.	%	Participants (359) No.	%
Trypanosoma cruzi	54	100.0	357	99.4
Parasite Screen	**Referees (58) No.**	**%**	**Participants (769) No.**	**%**
Blood flagellate, NOS, referred for identification	56	96.5	672	87.4
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	2	3.5	80	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 June 16, 2020. 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.

BP-08 Introduction

Thick and thin Giemsa-stained smears were obtained from a 58-year-old woman from Haiti with fever and lymphadenopathy. The specimen contained *Plasmodium falciparum*. A response of "*Plasmodium falciparum*", "*Plasmodium* sp., NOS, would refer or request another specimen, or perform additional molecular testing" and "*Plasmodium* sp./*Babesia* sp. seen, referred for identification" was considered satisfactory.

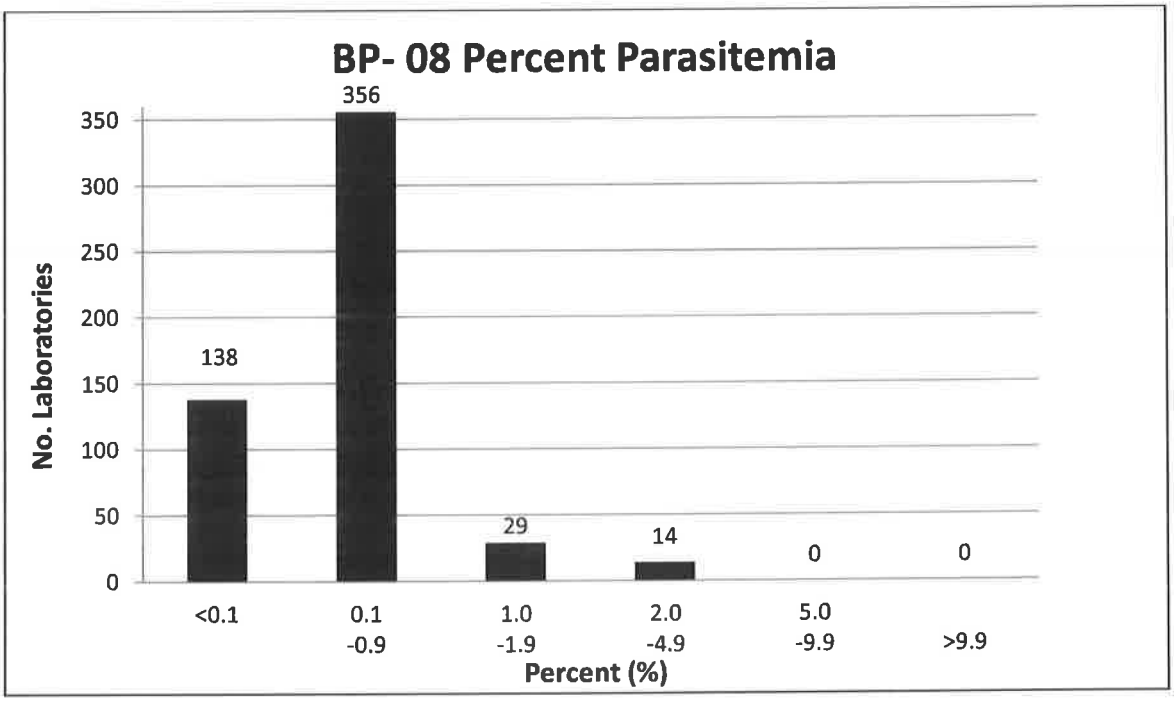
BP-08

Parasite Identification	Referees (54) No.	%	Participants (373) No.	%
Plasmodium falciparum	47	87.0	318	85.3
Plasmodium sp., NOS, would refer or request another specimen, or perform additional molecular testing	5	9.3	48	12.9

Parasite Screen	Referees (58) No.	%	Participants (758) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	51	87.9	702	92.6

BP-08

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)



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

- Normal size and shape of infected erythrocytes
- Smaller, more delicate ring-form trophozoites (approximately 1/5 the diameter of the erythrocyte) frequently with two chromatin dots (so-called "head phone" forms)
- Erythrocytes infected with multiple parasites
- Presence of ring-form trophozoites at the edge of the erythrocyte (appliqué forms)
- 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)
- Presence of crescent-shaped gametocytes (not always seen)
- 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

Distinguishing *P. falciparum* from *Babesia* spp. can be challenging, given that both have a predominance of small ring forms, infect RBCs of all ages, and there may be multiple parasites per erythrocyte. The presence of Maurer's clefts, malarial pigment, and cresent-shaped gametocytes eliminate *Babesia* infection from consideration. Furthermore, *Babesia* parasites are usually more pleomorphic with spindled, elliptical and ovoid forms. Extracellular forms are also more common in babesiosis. Finally, identification of the classic "Maltese cross" or tetrad form of *Babesia* sp. is diagnostic for babesiosis although it may rarely be observed. Molecular or antigen-detection methods, in addition to clinical/travel history may be useful adjuncts for distinguishing between these two similar appearing parasites.

Discussion

See discussion for specimen BP-06 on page 4.

BP-09 Introduction

A photo page of a Giemsa-stained blood smear obtained from a routine screening of an 8-year-old asylum seeker from the Democratic Republic of the Congo. 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.

BP-09

Parasite Identification	Referees (50) No.	%	Participants (322) No.	%
Microfilaria-*Mansonella* sp.	49	98.0	301	93.5
Microfilaria-*Wuchereria*	1	2.0	7	2.2

Parasite Screen	Referees (62) No.	%	Participants (808) No.	%
Microfilaria, NOS, referred for identification	61	98.4	737	91.2
Blood or tissue parasite, not *Plasmodium* sp./*Babesia* sp., referred for identification	1	1.6	67	8.3

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.

BP-10 Introduction

Blood sample were obtained from a 25-year-old female returning from an animal safari in Tanzania. The specimen contained *Trypanosoma brucei (gambiense* or *rhodesiense)*. A response of *"Trypanosoma brucei (gambiense* or *rhodesiense)"*, "Blood flagellate, NOS, referred for identification" or "Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification" was considered satisfactory.

BP-10

Parasite Identification	Referees (54) No.	%	Participants (359) No.	%
Trypanosoma brucei (*gambiense* or *rhodesiense*)	54	100.0	357	99.4
Parasite Screen	**Referees (58) No.**	**%**	**Participants (769) No.**	**%**
Blood flagellate, NOS, referred for identification	57	98.3	671	87.3
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	1	1.7	81	10.5

Discussion

Causal Agents

Human African trypanosomiasis (HAT) is caused by two subspecies of *Trypanosoma brucei, T. b. gambiense* (West and Central Africa) and *T. b. rhodesiense* (eastern and southeastern Africa). The type subspecies, *T. b. brucei*, does not cause human infection.

Biology and Life Cycle

Trypanosoma brucei spp. are transmitted by tsetse flies in the genus *Glossina*. When an infected tsetse fly takes a blood meal, metacyclic trypomastigotes are injected into the bloodstream where they transform into bloodstream trypomastigotes. There are two forms of bloodstream trypomastigotes, slender and stumpy. The slender trypomastigotes multiply by binary fission and perpetuate the blood cycle. Stumpy forms are adapted to be picked up by the tsetse fly vector. Within the midgut of the vector, stumpy forms develop into procyclic forms and multiply by binary fission. After a while, some procyclic forms leave the midgut and migrate via the hemocoel to the salivary glands, where they develop into epimastigote and eventually metacyclic forms. Metacyclic forms are non-dividing and are the infectious stage for the vertebrate host. Unlike with *T.cruzi*, there is no amastigote formation in the human host tissue, although *T. brucei* can cross the blood-brain barrier and cause central nervous system involvement.

Diagnosis

Diagnosis of *T. brucei* is made by the finding of trypomastigotes in blood, chancre fluid, lymph node aspirates, bone marrow, and CSF. A wet preparation may be examined for motility. Concentration techniques may increase the chances for a morphologic diagnosis, including centrifugation and examination of the buffy coat. Trypomastigotes (the only stage seen in the human host) are 14-33 µm long, have a large central nucleus, a small, terminal kinetoplast at the posterior end and a free flagellum leaving the body anteriorly. In stained blood films, it is possible to find diving forms, something not seen in cases with *T cruzi*. Currently, serologic, molecular, and rapid diagnostic (RDT) tests are not routinely available in the United States.

Clinical Significance

According to the World Health Organization in 2014, 3796 cases of HAT were reported. Human infection with *Trypanosoma brucei* presents with two clinical manifestations. In the first, the parasite is found in the peripheral blood and symptoms include fevers, headaches, malaise, and muscle and joint aches. In the second, parasites cross the blood-brain barrier to involve the central nervous system and can be found in the cerebrospinal fluid. During second stage disease, neurologic symptoms develop and mental status declines, eventually leading to coma and death. Disease progresses at different rates depending on which subspecies is involved, with *T. b. gambiense* having a more chronic, indolent course spanning years while *T. b. rhodesiense* progresses more rapidly over a period of months. If left untreated, both forms of African trypanosomiasis are fatal.

Treatment

All people with trypanosomal infection should be treated. First-line therapy depends on stage of disease and subspecies involved. For *T. b. gambiense* infections, pentamidine isethionate is the drug of choice for first-stage disease while combination therapy with nifurtimox and eflornithine is recommended for second-stage disease. For *T. b. rhodesiense* infections, suramin is the first-line treatment for first-stage disease while melarsoprol is recommended for second-stage disease. These therapies are generally effective yet have varying toxicity profiles. Of note, adverse reactions to melarsoprol can be severe and life-threatening with 5-18% of patients developing an encephalopathic reaction which is fatal in 10-70% of these patients.

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 June 16, 2020. http://www.cdc.gov/dpdx
3. Garcia LS. *Diagnostic Medical Parasitology*, 5th ed. ASM Press; 2007.

COLLEGE of AMERICAN PATHOLOGISTS

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We the participants below have completed the review of the CAP BP-B 2020 (Product Mailing, Year) Participant Summary/Final Critique report and can self-report the recommended 0.5 (Education Hours) hours towards fulfilling education and certification of maintenance requirements.

Participant	Date	Participant	Date

Director (or Designee) Signature - I have verified that the individuals listed above have successfully participated in this activity.	Date

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