Blood Parasite Identification

BP-01 Introduction

Thick and thin Giemsa-stained smears were obtained from a 43-year-old immigrant from Somalia with fever after returning home from a 1-month visit.The specimen contained *Plasmodium falciparum*. A response of "*Plasmodium falciparum*." or "*Plasmodium* sp./*Babesia* sp. seen, referred for identification" were considered satisfactory.

BP-01

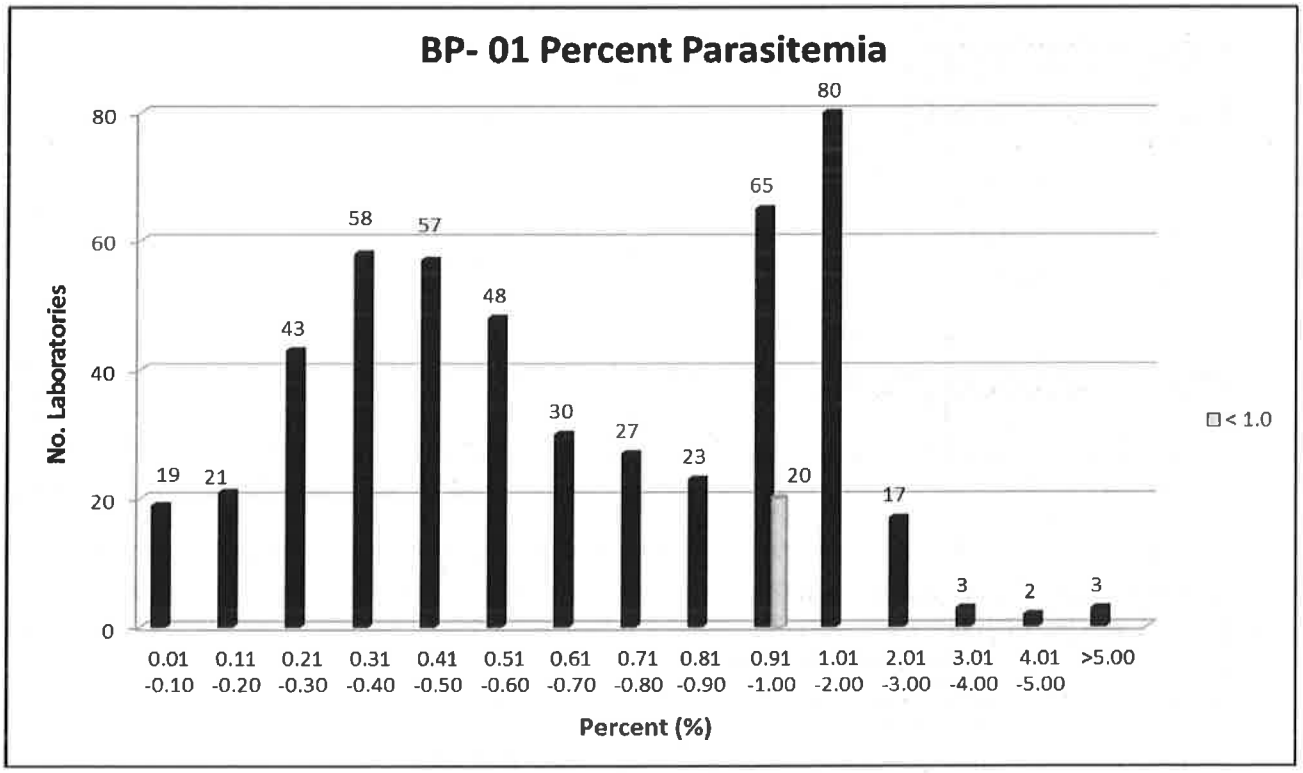
Parasite Identification*	Referees (29) No.	%	Participants (395) No.	%
Plasmodium falciparum	24	82.8	300	77.1
Plasmodium malariae	1	3.4	24	6.2
Plasmodium sp., not *P. falciparum*, referred for identification	2	6.9	39	10.0
Plasmodium vivax/ovale, NOS	2	6.9	15	3.9

Parasite Screen	Referees (15) No.	%	Participants (772) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	15	100.0	770	99.7

* Parasite identification graded by referee consensus

BP-01

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 stages (approximately 1/5 the diameter of the erythrocyte) frequently with two chromatin dots (so-called "head phone" forms)
- Erythrocytes infected with multiple ring forms
- Presence of ring forms at the edge of the erythrocyte (appliqué forms)
- Absence of mature trophozoites and schizonts in the peripheral blood film (may be seen if there is a delay in processing the blood specimen)
- 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 can produce multiply infected RBCs. The presence of Maurer's clefts, malarial pigment, and banana-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. Molecular or antigen-detection methods, in addition to clinical/travel history may be useful adjuncts for distinguishing between these two similar appearing parasites.

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: 1) many 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.

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. Available at: http://www.cdc.gov/malaria/diagnosis_treatment/clinicians3.html. Accessed June 23, 2015.
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. Available at: http://www.who.int/mediacentre/factsheets/fs094/en/ Accessed online October 26, 2015.
4. Garcia LS. *Diagnostic Medical Parasitology.* 5th ed. Washington, DC. 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-02 Introduction

Thick and thin Giemsa-stained smears were obtained from a 32-year-old immigrant from Panama who returns with fever. The specimen contained *Trypanosoma cruzi*. A response of "*Trypanosoma cruzi*", "Blood flagellate, NOS, referred for identification" and "Blood or tissue parasite, not Plasmodium sp. or Babesia sp., referred for identification" were considered satisfactory.

BP-02

Parasite Identification	Referees (32) No.	%	Participants (392) No.	%
Trypanosoma cruzi	32	100.0	388	99.0

Parasite Screen	Referees (12) No.	%	Participants (763) No.	%
Blood flagellate, NOS, referred for identification	12	100.0	631	82.7
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	-	-	102	13.4

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. 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.

BP-03 Introduction

Thick and thin Giemsa-stained smears were obtained from a 50-year-old male with fever and cervical adenopathy after returning from a missionary trip to northern Uganda. The specimen contained *Trypanosoma brucei*. A response of "*Trypanosoma brucei* (*gambiense* or *rhodesiense*)", "Blood flagellate, NOS, referred for identification" and "Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification" was considered satisfactory.

BP-03

Parasite Identification	Referees (32) No.	%	Participants (386) No.	%
Trypanosoma brucei (*gambiense* or *rhodesiense*)	31	96.9	381	98.7
Leishmania sp.	1	3.1	4	1.0
Parasite Screen	**Referees (12) No.**	**%**	**Participants (770) No.**	**%**
Blood flagellate, NOS, referred for identification	12	100.0	651	84.5
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	-	-	98	12.7

Discussion

Causal Agents

African trypanosomiasis 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 ssp. 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

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 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. 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. Garcia LS. 2007. *Diagnostic Medical Parasitology*, 5th ed., Washington, DC. ASM Press..

BP-04 Introduction

Thick and thin Giemsa-stained smears were obtained from an international traveler with fever, vomiting, and diarrhea after 2 weeks in South Africa. A response of "No parasite(s) seen" or "Specimen screened for blood parasites, no organisms seen" was considered satisfactory.

BP-04

Parasite Identification	Referees (31) No.	%	Participants (388) No.	%
No parasite(s) seen	31	100.0	384	99.0

Parasite Screen	Referees (12) No.	%	Participants (768) No.	%
Specimen screened for blood parasites, no organisms seen	11	91.7	760	99.0

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 10x 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 100X 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. 2007. *Diagnostic Medical Parasitology*, 5th ed., Washington, DC. ASM Press.
2. CDC: Malaria surveillance - United States 2008. *MMWR* 2010;59(SS07);1-15.
3. Greenwood BM, Bojang K, Whitty CJ, Targett GA. Malaria. *Lancet.* 2005;365:1487-1498.

BP-05 Introduction

A photograph challenge was obtained from an international adoptee from Cameroon with eosinophilia and transient skin swellings. The specimen contained Microfilaria-*Loa loa*. A response of "Microfilaria-*Loa loa*", "Microfilaria, NOS, referred for identification" and "Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification was considered satisfactory.

BP-05

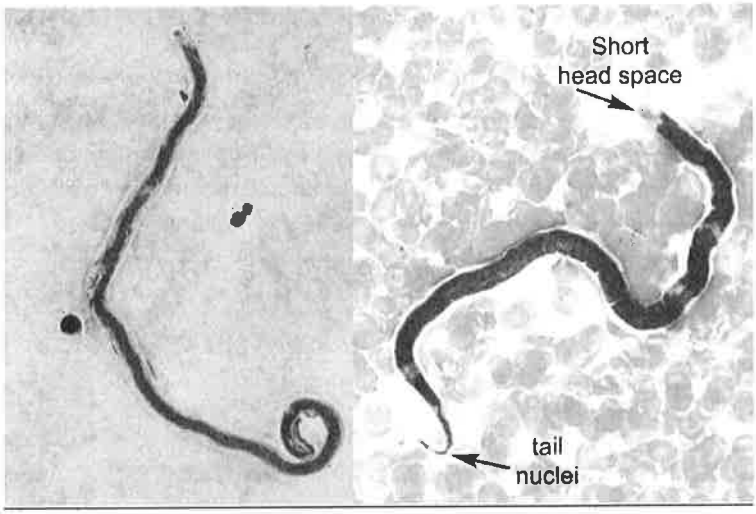
Parasite Identification*	Referees (31) No.	%	Participants (337) No.	%
Microfilaria-*Loa loa*	26	83.9	252	74.8
Microfilaria-*Onchocerca volvulus*	1	3.2	2	0.6
Microfilaria-*Mansonella* sp.	3	9.7	75	22.3
Parasite Screen	**Referees (13) No.**	**%**	**Participants (819) No.**	**%**
Microfilaria, NOS, referred for identification	13	100.0	754	92.1
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	-	-	58	7.1

* Parasite identification graded by referee consensus

Discussion

Key features that suggest an identification of *Loa loa* may include:

- Travel history to tropical Cameroon in Africa
- Presence of microfilariae in blood
- Size range of 231-250 micrometers
- Presence of a sheath (may not always be present in permanent stained specimens) that does not stain pink with Giemsa at a pH of 7.2
- Short head space
- Tapered tail with nuclei irregularly spaced to the tip of the tail.



Causal Agent

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.

COLLEGE of AMERICAN PATHOLOGISTS

Attestation of Participation for Self-Reported Training*

We the participants below have completed the review of the CAP BP-A 2016 (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

Retain this page for record-keeping and auditing purposes.
Individuals can also track their participation of educational activities through the CAP Learning Management System (LMS).

1. Create an account at cap.org\learning.
2. Enter your User ID and Password.
3. Under Personalized Options, select Training Transcript.
4. Select Learning Menu and choose Self-Reported Training.
5. Select New, enter the required information, and choose OK when complete.

For assistance, call our Customer Contact Center at 800-323-4040 or 847-832-7000 option 1.

**CAP Self-Reported Training activities do not offer CE credit, but can be used towards fulfilling requirements for certification of maintenance by agencies such as the American Society of Clinical Pathology (ASCP). Please verify with your certifying agency to determine your education requirements.*

49215