Blood Parasite Identification

BP-01 Introduction

Thick and thin Giemsa-stained smears were obtained from a blood transfusion recipient with cyclic fevers. The specimen contained *Plasmodium falciparum*. A response of "*Plasmodium falciparum*." or "*Plasmodium* sp., NOS would refer or request another specimen, or perform additional molecular testing" and "*Plasmodium* sp./*Babesia* sp. seen, referred for identification" were considered satisfactory.

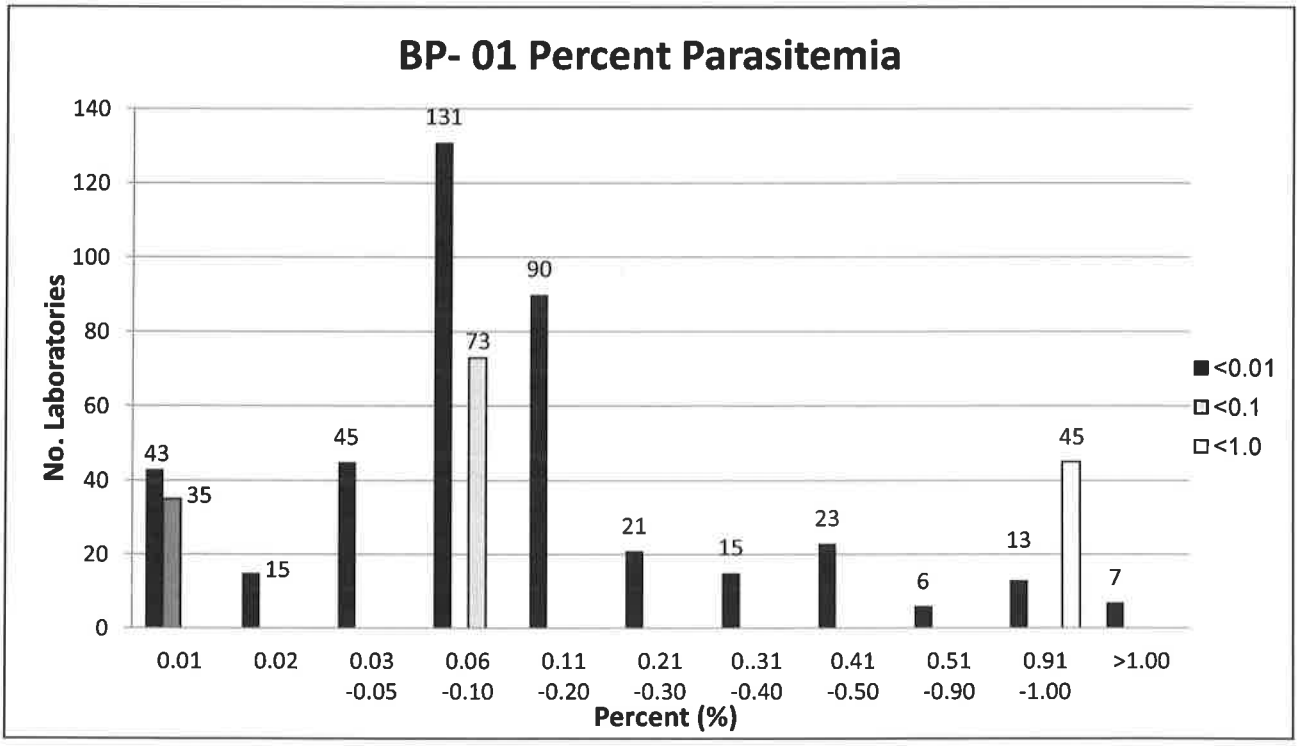
BP-01

Parasite Identification	Referees (29) No.	%	Participants (423) No.	%
Plasmodium falciparum	26	89.7	321	75.9
Plasmodium sp., NOS would refer or request another specimen, or perform additional molecular testing	1	3.5	58	13.7
Babesia sp.	2	6.9	16	3.8

Parasite Screen	Referees (19) No.	%	Participants (795) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	19	100.0	742	93.3

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 follow-up specimen on a patient from India recently diagnosed with malaria. The specimen contained Microfilaria-*Brugia* sp. A response of "Microfilaria-*Brugia* sp", "Microfilaria, NOS, referred for identification" and "Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification" was considered satisfactory.

BP-02

Parasite Identification	Referees (30) No.	%	Participants (355) No.	%
Microfilaria-*Brugia* sp	26	86.7	302	85.1
Microfilaria-*Wuchereria bancrofti*	4	13.3	47	13.2

Parasite Screen	Referees (18) No.	%	Participants (856) No.	%
Microfilaria, NOS, referred for identification	18	100.0	768	89.7
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	-	-	73	8.5

This case represented *Brugia malayi*. Diagnostic morphologic features that would support an identification of *Brugia* include:

- Large microfilaria (177-200 micrometers in length) that usually possess a sheath; in the case of *B. malayi* the sheath usually stains pink when stained with Giemsa at a pH of 6.8-7.2.
- A long head space
- A tapered tail with terminal and subterminal nuclei separated from the main nuclear column and each other by significant gaps.

The travel history in this case was also helpful, as *Brugia malayi* is endemic to India, east and south to Korea, the Philippines, and southern China. While the patient was noted as having been previously-diagnosed with malaria, there is less correlation between *Plasmodium* and *Brugia* as they are typically transmitted by different mosquito vectors; *Plasmodium* is transmitted by *Anopheles* mosquitoes while *B. malayi* is most commonly transmitted by *Mansonia* and *Aedes*. Co-infection between *Plasmodium* and *Wuchereria* is more common in endemic areas as they both utilize *Anopheles* as a vector.

Discussion

Causal Agent:
Lymphatic filariasis is caused by the filarid nematodes *Brugia malayi*, *B. timori*, and *Wuchereria bancrofti*. *Wuchereria bancrofti* is distributed nearly worldwide in the tropics. *Brugia malayi* is distributed in Southeast Asia and the Indian subcontinent, while *B. timori* is endemic to the Lesser Sunda Islands of the Indonesian archipelago.

Biology and Life Cycle:
All three species have a similar life cycle, and adults of all three species reside in the lymphatic vessels of the human definitive host. Gravid females release sheathed microfilariae which circulate in the blood at night, exhibiting nocturnal periodicity (except for some populations of *W. bancrofti* in Southeast Asia which do not express specific periodicity). An appropriate mosquito intermediate host becomes infected while ingesting microfilariae during the course of a blood meal. Microfilariae migrate from the midgut of the mosquito to the flight muscles where they develop into infective L3 larvae in approximately two weeks. L3 larvae migrate through the hemocoel of the mosquito to the head and mouthparts. Humans become infected when a mosquito deposits L3 larvae onto the skin while

taking a blood meal. Larvae migrate to the lymphatics system where it takes several months to develop into sexually mature adults.

Diagnosis:
The diagnosis of all three species is based primarily on the identification of microfilariae in thick and thin blood films stained with Giemsa, Wright stain, or hematoxylin. Concentration procedures, such the Knott's method, may increase sensitivity. Because all three species exhibit nocturnal periodicity, the optimal time to collect blood specimens from a patient is between 10 PM and 2 AM.

All three species may possess a sheath, although the sheath may be absent in stained blood smears so the absence of a sheath should not in itself rule-out any of these species. The most important features for identifying these nematodes to the genus level are the nuclear arrangements in the head and tail. The following table summarizes the important morphologic features.

Species	Size (in stained blood films)	Sheath (color, when properly stained with Giemsa)*	Head Space (distance between anterior end of nuclear column and tip of worm)	Tail nuclei
Wuchereria bancrofti	244-296 µm	Colorless*	Short	Tail anucleate
Brugia malayi	177-230 µm	Bright pink*	Long	Terminal and subterminal nuclei present, with gaps in between
Brugia timori	310 µm avg. length	Colorless*	Long	Terminal and subterminal nuclei present, with gaps in between

*Sheath color is pH-dependent, and at times the sheath of *B. malayi* may not stain bright pink. Likewise, on rare occasions, the sheath of *W. bancrofti* has been known to stain bright pink.

There are no routine molecular or rapid tests available for lymphatic filariasis in the United States. A rapid format immunochromatographic test is available outside the U.S. however. An EIA is available for detecting circulating antibodies in blood. Unlike with microscopy, blood does not need to be collected at night to perform the EIA. This test is reliable for *W. bancrofti* and *B. malayi*, but has not been properly validated for *B. timori*. There is also some cross-reactivity with *Onchocerca volvulus* and *Loa loa*.

Clinical Significance:
Most microfilarial infections are asymptomatic with subclinical tortuosity and dilation of lymphatics. The spectrum of disease for those with symptoms includes lymphedema, hydrocele, acute attacks of febrile lymphangitis and, less frequently, pulmonary tropical eosinophilia syndrome or chyluria. The range of clinical presentations varies slightly with species and geography. For example, involvement of the genital lymphatics occurs almost exclusively with *W. bancrofti* infection. Acute symptoms are often more intense in patients from non-endemic areas. With low worm burden and a good immune response, long-term sequelae in these patients are rare. In contrast, for those who live in endemic areas and sustain repeated bites by infected mosquitos, worm burdens are higher and lymphatics are more likely to become obstructed leading to chronic lymphedema. Lymphedema occurs more commonly in the lower extremities but can also involve the upper extremities, breasts in females and scrotum in males. Subsequent skin thickening and fissuring invites recurrent bacterial infection. With time, the lymphedema and skin changes can progress to elephantiasis.

Treatment:

The treatment of choice for active lymphatic filariasis is diethylcarbamazine (DEC) because it is both microfilaricidal and active against the adult worm. Adult worms must be killed in order to prevent relapse. However, DEC is contraindicated in patients with onchocerciasis co-infection and should be used with extreme caution in those with loa loa infections. There is also some evidence that treatment targeting *Wolbachia*, the rickettsial endosymbiont bacteria that lives inside *Wuchereria* and *Brugia* spp., may stop microfilarial production. Due to low prevalence of the disease, DEC is no longer FDA-approved in the United States but can be obtained through the Centers for Disease Control and Prevention. Other therapeutic options include ivermectin (kills only microfilariae), and albendazole (has some macrofilarial activity). If lymphedema is already established, antifilarial medication has not been shown to be of benefit. Instead, management of symptoms includes exercise, elevation and local skin care.

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
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BP-03 Introduction

Thick and thin Giemsa-stained smears were obtained from a 3-year-old adoptee from Ethiopia. The specimen contained *Plasmodium vivax*. A response of "*Plasmodium vivax*", "*Plasmodium vivax/ovale*, NOS", "*Plasmodium* sp., not *P. falciparum*, referred for indentification", and "*Plasmodium* sp., NOS would refer or request another specimen, or perform additional molecular testing" was considered satisfactory.

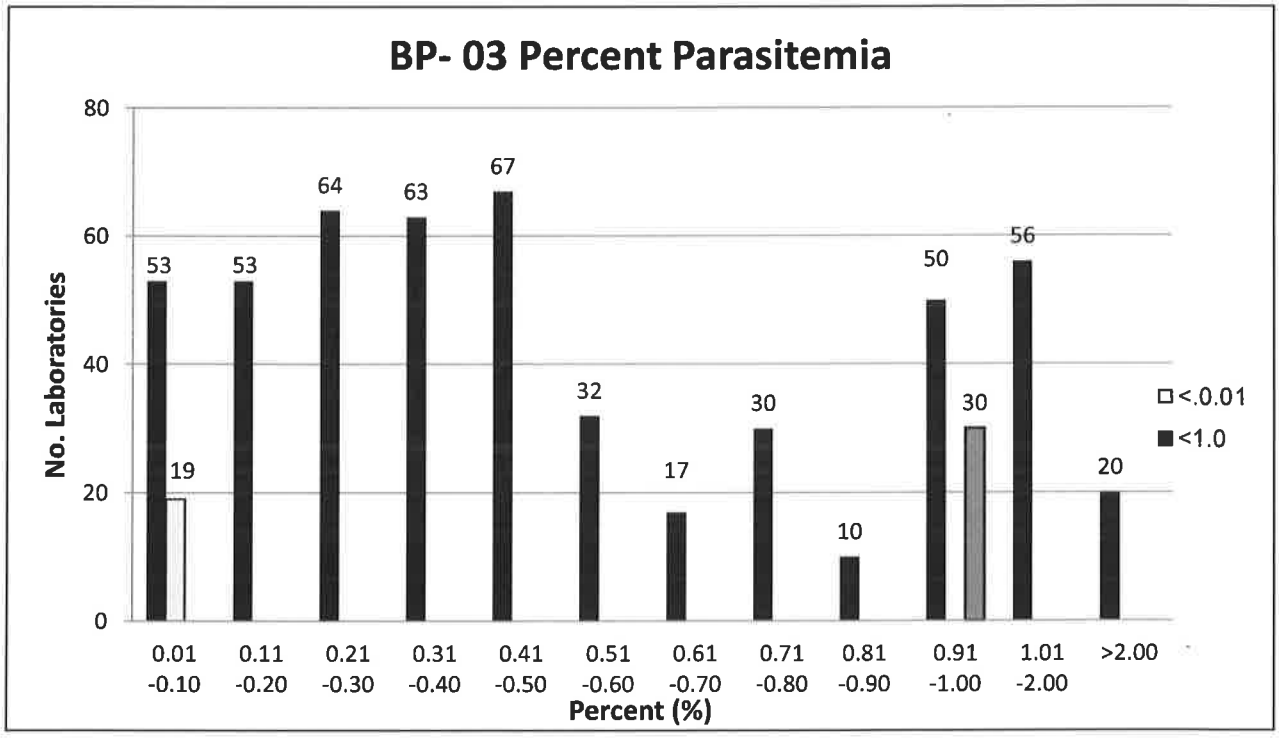
BP-03

Parasite Identification	Referees (30) No.	%	Participants (442) No.	%
Plasmodium vivax	16	53.3	224	50.7
Plasmodium vivax/ovale, NOS	5	16.7	83	18.8
Plasmodium sp., not *P. falciparum*, referred for identification	7	23.3	95	21.5
Plasmodium sp., NOS would refer or request another specimen, or perform additional molecular testing	-	-	11	2.5
Plasmodium ovale	2	6.7	12	2.1

Parasite Screen	Referees (18) No.	%	Participants (775) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	18	100.0	769	99.2

BP-03

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)



In the case of an actual patient specimen, both thick and thin blood films should be prepared for evaluation of malaria. 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. The travel history in this case was also supportive, as *P. vivax* is one of the most prevalent species found in Ethiopia (30-40%), second only to *P. falciparum* (60-70%). Both *P. ovale* and *P. malariae* are considered rare in Ethiopia.

Key morphologic features on a thin blood film that would suggest the diagnosis of *P. vivax* are:

- Enlarged size of the infected RBCs compared to the uninfected cells.
- The ring-form trophozoites usually with sturdy cytoplasm and one or two (often more commonly one) chromatin dots.
- Developing trophozoites ameboid.
- Gametocytes round to pleomorphic (in the latter, may appear to 'hug' surrounding RBCs).
- Schüffner's dots are typically present in all cells except early ring forms, when stained with Giemsa at a pH of.8-7.2.
- Mature schizonts with 12-24 merozoites.
- All stages are usually present.

Discussion

See discussion for BP-01 on page 3

BP-04 Introduction

Thick and thin Giemsa-stained smears were obtained from a heart transplant recipient with fever, anorexia, and mild lymphadenopathy. 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-04

Parasite Identification	Referees (30) No.	%	Participants (406) No.	%
Trypanosoma cruzi	30	100.0	398	98.0
Parasite Screen	**Referees (18) No.**	**%**	**Participants (805) No.**	**%**
Blood flagellate, NOS, referred for identification	17	94.4	692	86.0
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	1	5.6	98	12.2

This case simulated a *T. cruzi* infection in a heart transplant recipient. There are two main clinical scenarios in heart-transplant recipients concerning Chagas disease. One involves reactivation in patients harboring tissue amastigotes due to a prior infection. The amastigotes transform into blood trypomastigotes when the patient is immunosuppressed to receive the transplanted heart (or other organs). Another possibility is that the donor heart was infected with amastigotes which transformed into circulating trypomastigotes while the patient was immunosuppressed.

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 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 cardiac (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

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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-05 Introduction

Thick and thin Giemsa-stained smears were obtained from a 27-year-old male returning from Africa with fever, hematuria, and intestinal distress.. A response of "No parasite(s) seen" or "Specimen screened for blood parasites, no organisms seen" was considered satisfactory.

BP-05

Parasite Identification	Referees (29) No.	%	Participants (403) No.	%
No parasite(s) seen	29	100.0	402	99.8
Parasite Screen	**Referees (19) No.**	**%**	**Participants (811) No.**	**%**
Specimen screened for blood parasites, no organisms seen	19	100.0	791	97.5

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

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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.