Blood Parasite Identification

BP-01 Introduction

Thick and thin Giemsa-stained smears were obtained from a 27-year-old female flight attendant returning from Nigeria. 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.

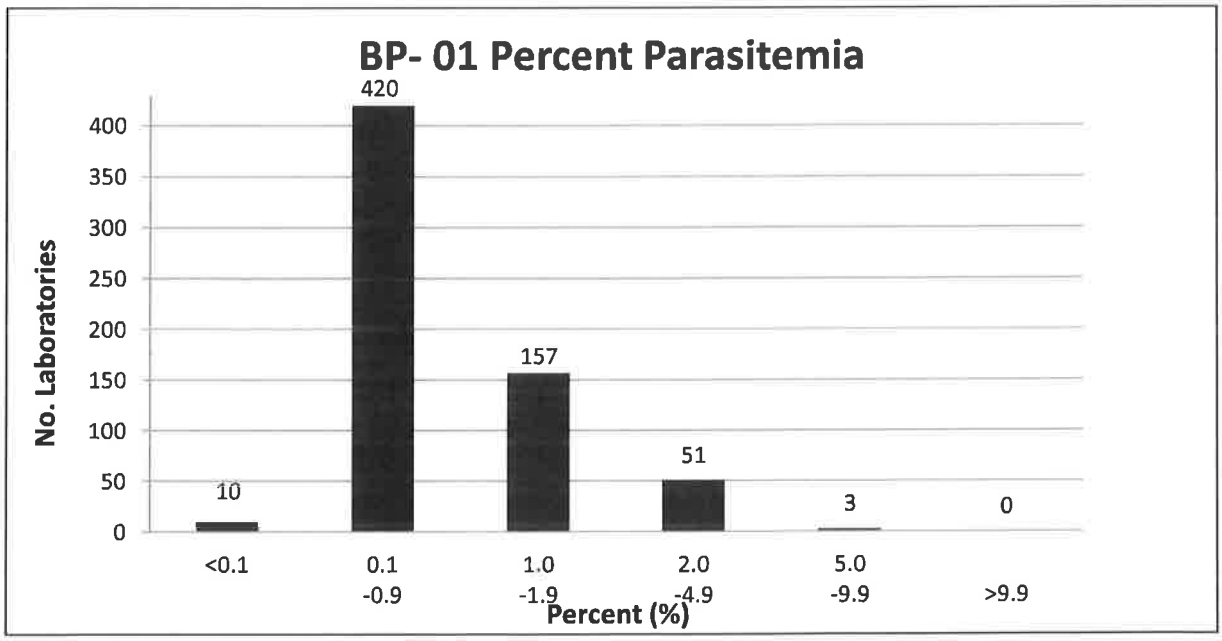
BP-01

Parasite Identification	Referees (48) No.	%	Participants (470) No.	%
Plasmodium falciparum	33	68.8	336	71.5
Plasmodium sp., NOS, would refer or request another specimen, or perform additional molecular testing	10	20.8	99	21.1
Plasmodium sp., not *P. falciparum*, referred for identification	1	2.1	13	2.8
Plasmodium malariae	2	4.2	11	2.3
Plasmodium vivax/ovale, NOS	2	4.2	9	1.9

Parasite Screen	Referees (58) No.	%	Participants (782) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	58	100.0	780	99.7

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-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

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. Available at: http://www.cdc.gov/malaria/diagnosis_treatment/clinicians3.html. Accessed February 21, 2020.
2. World Health Organization. Guidelines for the Treatment of Malaria. 3rd ed. Geneva, 2015.
3. World Health Organization. Malaria: Fact Sheet #94. Updated March 2019. Available at: http://www.who.int/mediacentre/factsheets/fs094/en/ Accessed online February 21, 2020.
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 41-year-old relief worker in India with generalized lymphadenopathy. 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" would have been considered satisfactory.

BP-02

Parasite Identification	Referees (43) No.	%	Participants (393) No.	%
Microfilaria-*Brugia* sp.	42	97.7	349	88.8
Microfilaria-*Wuchereria bancrofti*	1	2.3	35	8.9

Parasite Screen	Referees (63) No.	%	Participants (858) No.	%
Microfilaria, NOS, referred for identification	61	96.8	772	90.0
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	2	3.2	69	8.0

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
3. Garcia LS. 2007. *Diagnostic Medical Parasitology*, 5th ed., Washington, DC. ASM Press.
4. Chatterjee S, Nutman TB. "Filarial Nematodes." In *Manual of Clinical Microbiology,* ed. Jorgensen JH et al., 2461-2470. Washington, DC. ASM Press, 2015.

BP-03 Introduction

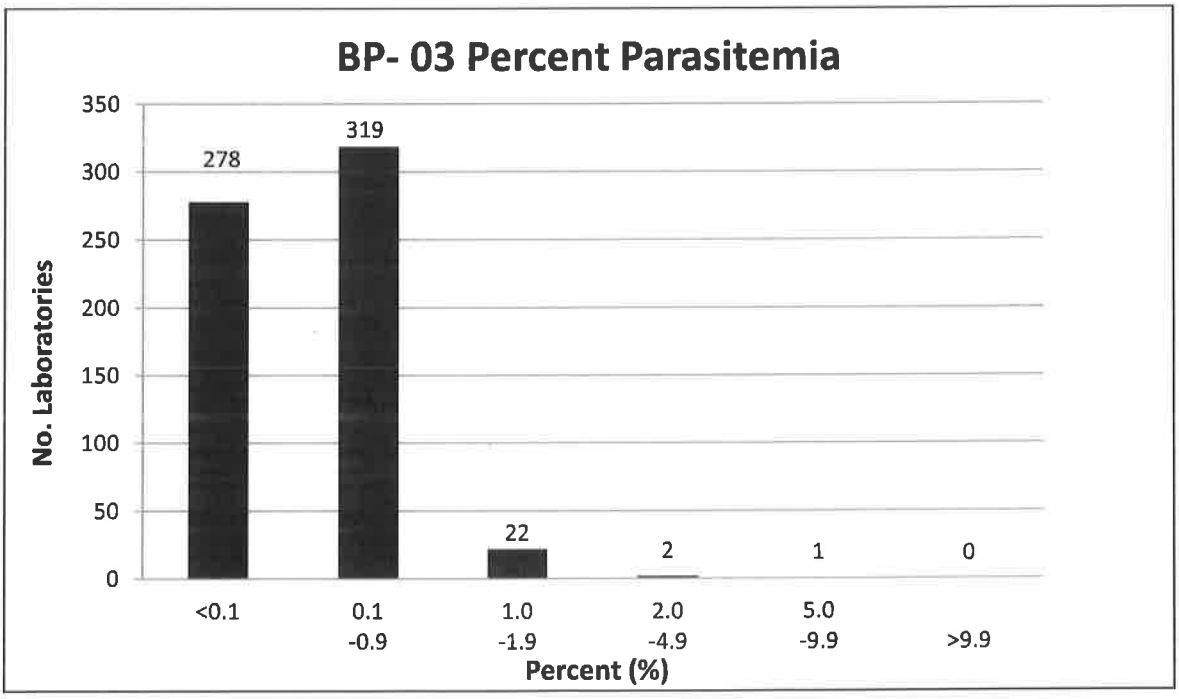
Thick and thin Giemsa-stained smears were obtained from a 50-year-old woman returning from visiting family in Rwanda. The specimen contained *Plasmodium malariae*. A response of " *Plasmodium malariae*", " *Plasmodium* sp., not *P. falciparum*, referred for identification", "*Plasmodium* sp., NOS, would refer or request another specimen, or perform additional molecular testing", and "*Plasmodium* sp./*Babesia* sp.seen, referred for identification" was considered satisfactory.

BP-03

Parasite Identification	Referees (48) No.	%	Participants (475) No.	%
Plasmodium malariae	23	47.9	195	41.0
Plasmodium sp., not *P. falciparum*, referred for identification	13	27.1	141	29.7
Plasmodium sp., NOS, would refer or request another specimen, or perform additional molecular testing	9	18.8	76	16.0
Plasmodium vivax/ovale, NOS	2	4.2	29	6.1
Parasite Screen	**Referees (58) No.**	**%**	**Participants (776) No.**	**%**
Plasmodium sp./*Babesia* sp. seen, referred for identification	56	96.5	737	95.0

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)



Identification

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

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

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

Life Cycle

The vector for malaria is the female anopheline mosquito, where the infected mosquito injects sporozoites after a blood meal. Sporozoites are carried via the blood to the liver where they invade hepatocytes and undergo an exoerythrocytic cycle with the formation of liver schizonts. The liver schizonts rupture, releasing large numbers of merozoites that then invade erythrocytes starting the erythrocytic cycle. In the infected erythrocyte, early rings (trophozoites) develop and mature into schizonts. The mature schizont contains merozoites that are then released into the bloodstream. While most merozoites proceed to infect other erythrocytes, a few develop into male and female gametocytes that are capable of infecting mosquitoes. There is no resting stage in the liver.

Clinical Relevance

Plasmodium malariae occurs less commonly than *P. falciparum* and *P. vivax* compromising 2.0% of malaria cases reported in the United States. A high percentage of *P. malariae* cases occur in West Africa. Unlike other *Plasmodium* species, the cyclic fevers for *P. malariae* occur every 72 hours and the clinical manifestations are usually mild due to lower parasitemia. No resistance to antimalarials has been established.

References

1. Garcia LS. *Diagnostic Medical Parasitology*, 5th ed. Washington, DC: ASM Press, 2007.
2. CDC: Malaria surveillance – United States 2007. *MMWR* 2009;58(SS02);1-16.
3. Guerrant RL, Walker DH, Weller PF. *Tropical Infectious Diseases*, Philadelphia: Churchill Livingstone. 1999.
4. Gonzalez-Garcia JJ, Arnalich F, Pena JM, et al. *An outbreak of Plasmodium vivax malaria among heroin users in Spain. Trans R Soc Trop Med Hyg.* 1986;80:549-552.

BP-04 Introduction

Thick and thin Giemsa-stained smears were obtained from 25-year-old soldier returning from Afghanistan with recurrent fevers. A response of "No parasite(s) seen" or "Specimen screened for blood parasites, no organisms seen" was considered satisfactory.

BP-04

Parasite Identification	Referees (48) No.	%	Participants (449) No.	%
No parasite(s) seen	48	100.0	443	98.7

Parasite Screen	Referees (58) No.	%	Participants (805) No.	%
Specimen screened for blood parasites, no organisms seen	57	98.3	784	97.4

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

BP-05 Introduction

Thick and thin Giemsa-stained smears were obtained from a 49-year-old hunter returning from his cabin in Minnesota. The specimen contained *Babesia* species. A response of "*Babesia* sp." or "*Plasmodium* sp./*Babesia* sp. seen, referred for identification" was considered satisfactory.

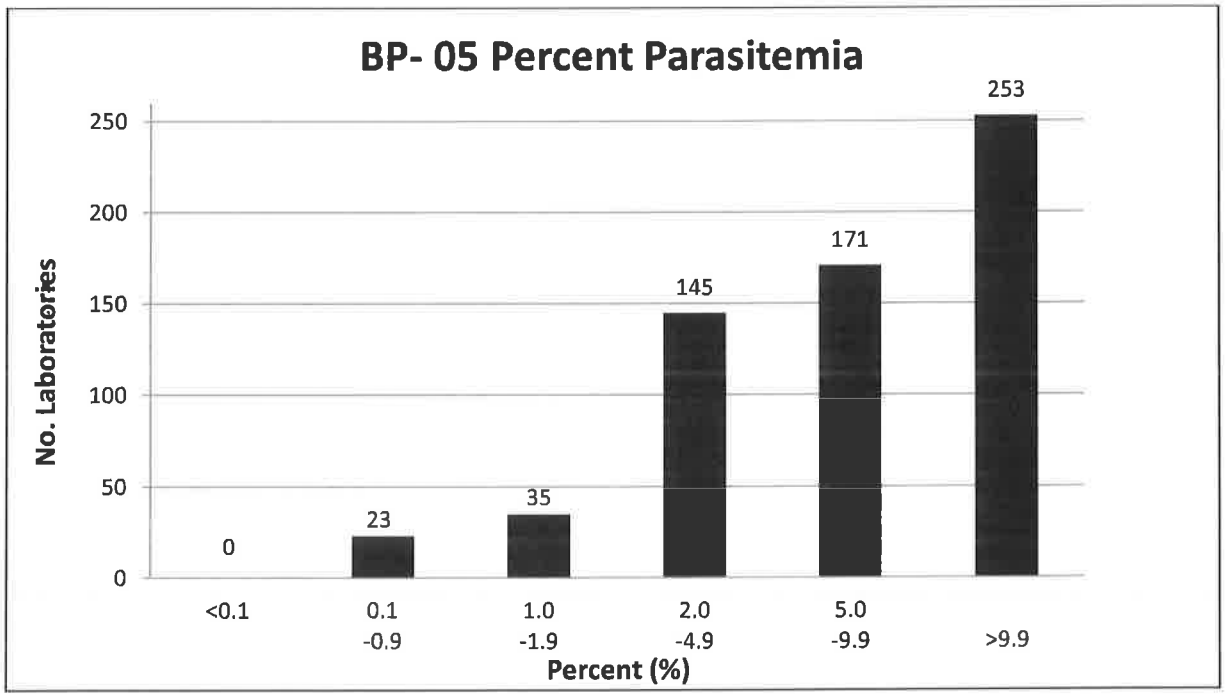
BP-05

Parasite Identification	Referees (48) No.	%	Participants (477) No.	%
Babesia sp.	48	100.0	467	97.9

Parasite Screen	Referees (58) No.	%	Participants (776) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	57	98.3	753	97.0

BP-05

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)



Summary of Key Features for Identification:

- *Babesia* spp. infect red blood cells of all sizes.
- Only the ring stages are identified on blood smears.
- Differentiating *Babesia* spp. from *P. falciparum* is sometimes difficult. The distinguishing features of *Babesia* spp. include pleomorphic ring forms, the "Maltese cross pattern" and extracellular forms.
- Unlike *Plasmodium* species, *Babesia* species never produce pigment.

Upon initial presentation of the patient, the parasite may present in such low numbers in blood that they cannot be seen in thin films. The diagnosis then depends on finding parasites in the thick films. If only ring forms are present on the thick films, it may be impossible to distinguish between *P. falciparum* and *Babesia* species. The clinical history along with collection of multiple blood samples then are of extreme importance. Molecular methods (PCR) testing or malarial antigen testing may also be useful in these cases. Confirmed and suspected cases should be reported to the department of health.

Discussion

Causal Agents

Babesiosis is caused by apicomplexan parasites of the genus *Babesia*. Species most-commonly attributed to human disease include *B. microti* (endemic to northeastern and northern midwestern United States; introduced elsewhere), *B. duncani* (northwestern North America), *B. divergens* (Old World), and *Babesia* sp. MO-1 (sometimes referred to as *Babesia divergens*-like, midwestern United States).

Biology and Life Cycle

Babesia spp. have a two-host life cycle. The definitive hosts are ticks in the genus *Ixodes*, while the intermediate hosts are mammals (usually rodents in nature). Infective sporozoites are released into the mammalian hosts when an infected *Ixodes* takes a blood meal. Sporozoites enter erythrocytes and undergo asexual reproduction by budding. Early ring-form trophozoites give rise to merozoites (including the classic 'tetrad form') that either produce more trophozoites or gametes. The latter is a dead-end stage in the mammalian host, and gametes must be ingested by a tick in order for sexual reproduction to occur. Humans can become infected either by being fed upon by an infected tick or through blood transfusions. Rare congenital cases have also been reported.

Diagnosis

Human babesiosis is typically diagnosed initially by the finding of trophozoites and merozoites on stained blood films. Merozoites displayed in a 'Maltese-cross' formation (tetrads) are typically diagnostic for *Babesia* spp., but are not commonly observed. Trophozoites maybe ring-shaped, pyriform (pear-shaped), or pleomorphic and vacuolated, and arranged singly or in short chains. Multiply-infected RBCs are not uncommon. Ring-form trophozoites need to be distinguished from *Plasmodium* spp., especially *P. falciparum*. *Plasmodium* spp. produce pigment, which is never produced by *Babesia* spp. In cases of babesiosis, extracellular ring-forms may be observed, singly or in clusters.

Species-level identification of *Babesia* cannot be accomplished by microscopy alone. Patient travel history can be useful information for determining the species of *Babesia* present, but confirmatory diagnosis should be performed by molecular (PCR, or PCR in combination with sequencing analysis) or serologic methods. Serology can also play a very important role in screening potential donors in transfusion-acquired cases.

Clinical significance

The clinical outcome can vary from asymptomatic infection to death depending on infective organism, presence of other tick-borne infections such as borreliosis, age, host immune status, and other underlying factors (eg, splenectomy or a history of blood transfusions). Presenting symptoms include malaise, chills, myalgia, fatigue, anemia, and high-grade fever not unlike acute malaria. More chronic symptoms described in cases are nausea, vomiting, night sweats, loss of weight, and bloody or dark urine.

Treatment

The majority of babesiosis cases self-resolve without need for drug therapy. The standard of care for more severe cases is clindamycin combined with quinine. An alternative regimen is atovaquone combined with azithromycin. In severe disease, or in splenectomized patients, exchange transfusion may be needed in addition to antimicrobial therapy.

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