Blood Parasite Identification

BP-01 Introduction

Thick and thin Giemsa-stained smears were obtained from routine screening of 43-year-old woman from India. The specimen contained *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-01

Parasite Identification	Referees (45) No.	%	Participants (372) No.	%
Microfilaria-*Brugia* sp	41	91.1	300	80.7
Microfilaria-*Wuchereria bancrofti*	2	4.4	55	14.8

Parasite Screen	Referees (65) No.	%	Participants (856) No.	%
Microfilaria, NOS, referred for identification	61	93.8	718	83.9
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	1	1.5	68	7.9

Discussion

Causal Agents

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 as 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.

Table 1.
Comparison of the human microfilariae found in blood specimens.

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

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-02 Introduction

Thick and thin Giemsa-stained smears were obtained from a 55-year-old male from Europe with hemolytic anemia. The specimen contained *Babesia* species. A response of "*Babesia* sp." or "*Plasmodium* sp./*Babesia* sp. seen, referred for identification" was considered satisfactory.

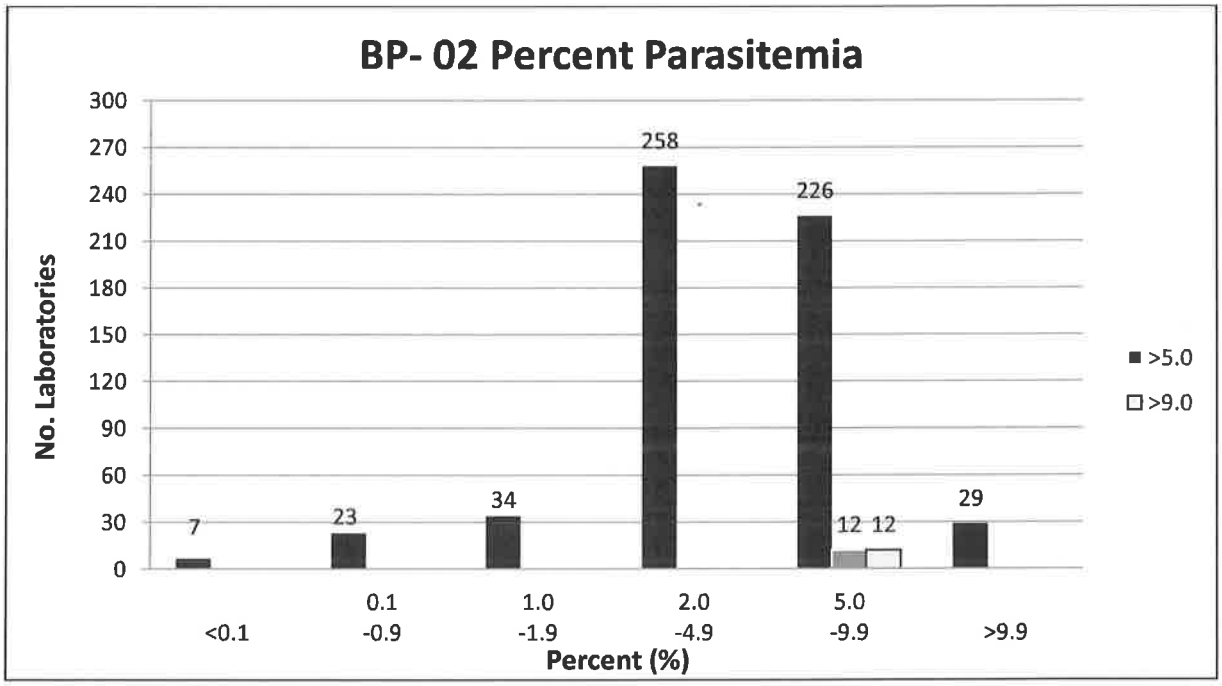
BP-02

Parasite Identification	Referees (49) No.	%	Participants (434) No.	%
Babesia sp.	46	93.9	399	91.9
Plasmodium sp., NOS would refer or request another specimen, or perform additional molecular testing	1	2.0	7	1.6
Plasmodium falciparum	1	2.0	21	4.8
Plasmodium sp., not *P. falciparum*, referred for identification	1	2.0	4	0.9

Parasite Screen	Referees (61) No.	%	Participants (795) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	59	96.7	777	97.7
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	1	1.6	2	0.3

BP-02

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 (e.g., 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.

References

1. Ash LP, Orihel TC. *Atlas of Human Parasitology*, 5th ed. Chicago, IL: ASCP Press: 2007.
2. DPDx-Laboratory Diagnosis of Parasites of Public Health Concern. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/dpdx/ [accessed March 4, 2019]
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
4. Westblade LF, Simon MS, Mathison BA, Kirkman LA. *Babesia microti*: from mice to ticks to an increasing number of highly susceptible humans. *J Clin Microbiol.* 2017;55:2903-2912

BP-03 Introduction

Thick and thin Giemsa-stained smears were obtained from a 25-year-old female from Pennsylvania recently returned from visiting family in Uganda. 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" were considered satisfactory.

BP-03

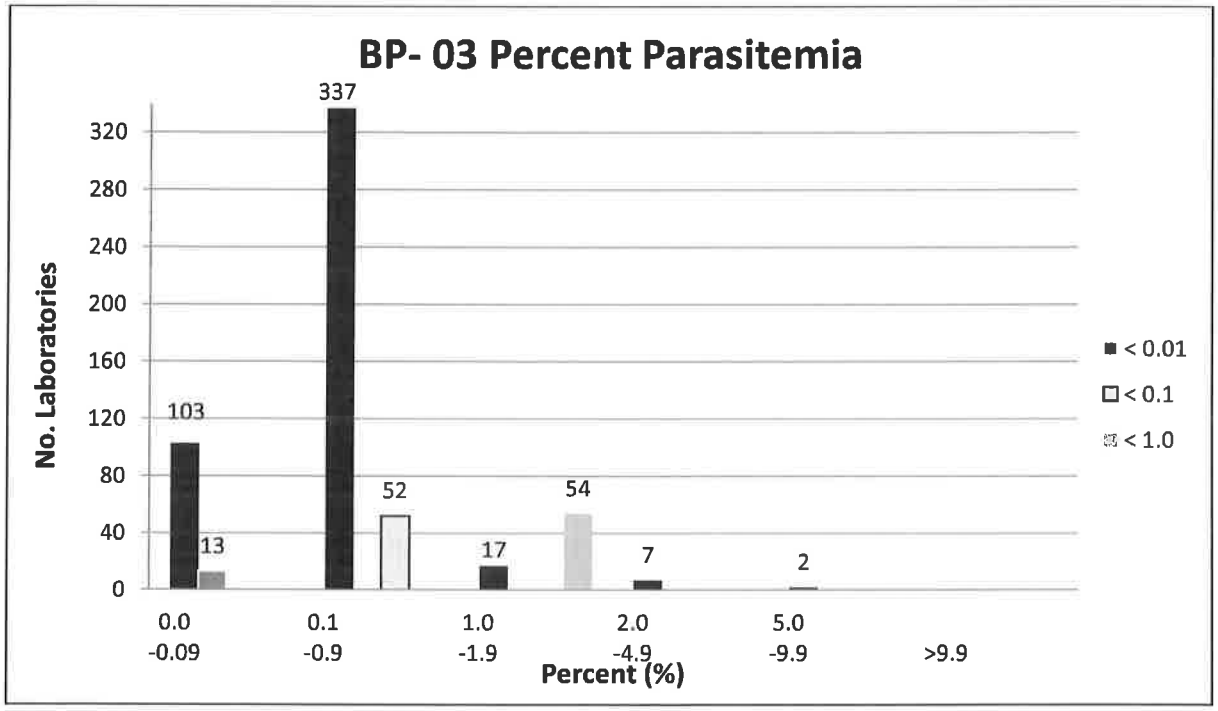
Parasite Identification	Referees (49) No.	%	Participants (425) No.	%
Plasmodium falciparum	30	61.2	226	53.2
Plasmodium sp., NOS would refer or request another specimen, or perform additional molecular testing	12	24.5	109	25.6
Plasmodium malariae	3	6.1	39	9.2
Plasmodium sp., not *P. falciparum*, referred for identification	3	6.1	30	7.1
Plasmodium vivax/ovale, NOS	1	2.0	10	2.4

Parasite Screen	Referees (61) No.	%	Participants (804) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	58	95.1	766	95.3

Parasite Identification graded by referee consensus.

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)



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 7.0-7.2

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*. *Plasmodium ovale* is sometimes divided into two subspecies which may actually represent valid species, *P. o. walkeri* and *P. o. curtisi*. In addition, there are at least six species of simian *Plasmodium* that have been documented to cause zoonotic infections in humans, the most notable being *P. knowlesi*, human infections of which appear to be increasing on the Malaysian peninsula (see also Table 1).

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

Table 1.
Plasmodium species recorded infecting humans, their geographic distributions, and natural intermediate (mammalian) hosts.

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

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

Biology and Life Cycle

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

Diagnosis

General Considerations

Malaria is primarily diagnosed by the identification of *Plasmodium* parasites on thin and thick blood films stained with Giemsa, Wright, or Wright-Giemsa stain. Molecular methods such as PCR may be employed when an

Calculating Percent Parasitemia

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

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

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

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

Procedural notes:

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

Important parasitemia thresholds

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

Molecular Detection

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

Table 3.
The following table highlights the advantages and disadvantages for the molecular detection of *Plasmodium* species (adapted from Mathison and Pritt 2017)

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

Antigen Detection

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

Antibody Detection

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

Clinical Significance

Nearly half of the world's population is at risk of malaria. In 2017, there were an estimated 219 million cases of malaria in 90 countries, with 435,000 deaths. Most malaria cases (92%) and deaths (93%) occur sub-Saharan Africa. There are approximately 1,000 cases of malaria diagnosed in the United States each year, almost all in recent travelers and immigrants. The visiting friends and relatives (VFR) population contribute to the vast majority of cases in travelers returning to non-endemic areas. Although the *Anopheles* mosquito is endemic in parts of North America, malaria transmission was largely eliminated in the 1940s through public health efforts.

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

Treatment

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

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

References

1. Centers for Disease Control and Prevention. Treatment of Malaria: Guideline for Clinicians. http://www.cdc.gov/malaria/diagnosis_treatment/clinicians3.html. Accessed online January 23, 2019.
2. Greenwood BM, Bojang K, Whitty CJ, Targett GA. Malaria. *Lancet*. 2005;365:1487-1498.
3. Mace KE, Arguin PM, Tan KR. Malaria surveillance – United States 2015. *Morbidity and Mortality Weekly Report*. 2018;67:1-28.
4. Mathison BA, Pritt BS. Update on malaria diagnostics and test utilization. *J Clin Microbiol*. 2017;55:2009-2017.
5. World Health Organization. Guidelines for the Treatment of Malaria. Second edition. Geneva, 2010.
6. World Health Organization. Malaria: Fact Sheet. Updated November 2018. https://www.who.int/news-room/fact-sheets/detail/malaria Accessed online January 23, 2019.

BP-04 Introduction

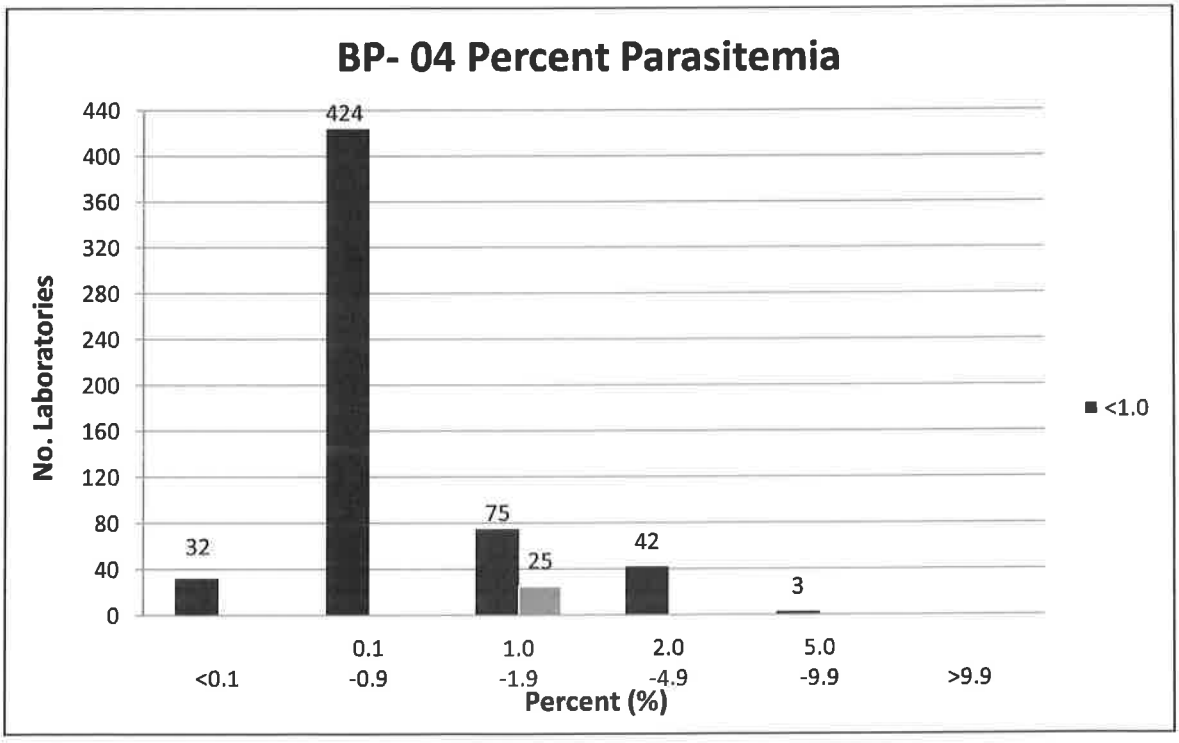
Thick and thin Giemsa-stained smears were obtained from a 65-year-old veteran with cyclic fevers and a history of malaria. The specimen contained *Plasmodium* vivax. A response of "*Plasmodium vivax*", "*Plasmodium vivax/ovale*, NOS","*Plasmodium* sp., not *P. falciparum*, referred for identification","*Plasmodium* sp., NOS would refer or request another specimen, or perform additional molecular testing", and "*Plasmodium* sp./*Babesia* sp.seen, referred for identification" was considered satisfactory.

BP-04

Parasite Identification	Referees (50) No.	%	Participants (445) No.	%
Plasmodium vivax	16	32.0	174	39.1
Plasmodium vivax/ovale, NOS	20	40.0	150	33.7
Plasmodium sp., not *P. falciparum*, referred for identification	8	16.0	75	16.9
Plasmodium sp., NOS would refer or request another specimen, or perform additional molecular testing	2	4.0	22	4.9
Plasmodium malariae	3	6.0	14	3.1
Parasite Screen	**Referees (60) No.**	**%**	**Participants (786) No.**	**%**
Plasmodium sp./*Babesia* sp. seen, referred for identification	60	100.0	780	99.2

BP-04

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. In this specimen the primary challenge was the identification of *P. vivax*.

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

1. Enlarged size of the infected RBCs compared to the uninfected cells.
2. The ring-form trophozoites usually with sturdy cytoplasm and one or two (often more commonly one) chromatin dots.
3. Developing trophozoites ameboid.
4. Gametocytes round to pleomorphic (in the latter, may appear to 'hug' surrounding RBCs).
5. Schüffner's dots are typically present in all cells except early ring forms, when stained with Giemsa at a pH of 7.0-7.2.
6. Mature schizonts with 12-24 merozoites.
7. All stages are usually present.

Discussion

Please refer to discussion starting on page 9.

BP-05 Introduction

Thick and thin Giemsa-stained smears were from a 40-year-old transplant recipient originally from Honduras. 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-05

Parasite Identification	Referees (50) No.	%	Participants (426) No.	%
Trypanosoma cruzi	49	98.0	422	99.1
Leishmania sp.	1	2.0	3	0.7

Parasite Screen	Referees (60) No.	%	Participants (803) No.	%
Blood flagellate, NOS, referred for identification	58	96.7	693	86.3
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	2	3.3	96	12.0

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 infects 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 6 and 7 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

COLLEGE of AMERICAN PATHOLOGISTS

Attestation of Participation of Self-Reported Training*

We the participants below have completed the review of the CAP BP-A 2019 (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.

1. Go to www.cap.org
2. Click **LOG IN / LOG IN** and enter your User ID and Password.
   - If you are unsure whether you have an *individual* web account with the CAP, or do not remember your user ID and password, click on **PASSWORD HINT**.
   - If you do not have an *individual* web account, click **CREATE AN ACCOUNT**. Complete and submit the account request form. You will be notified within one business day that your individual account has been activated.
3. Click **Learning** from the top menu bar
4. Click **Learning Transcript** from the menu bar
5. Click **Add My Own Activity**
6. Follow prompts to enter 'Self-Reported Training Activities'.

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.*