Blood Parasite Identification

BP-11 Introduction

Thick and thin Giemsa-stained smears were obtained from a 55-year-old gardener from New England with malaise and low-grade fever. The specimen contained *Babesia* species. A response of "*Babesia* sp." or "*Plasmodium* sp./*Babesia* sp. seen, referred for identification" was considered satisfactory.

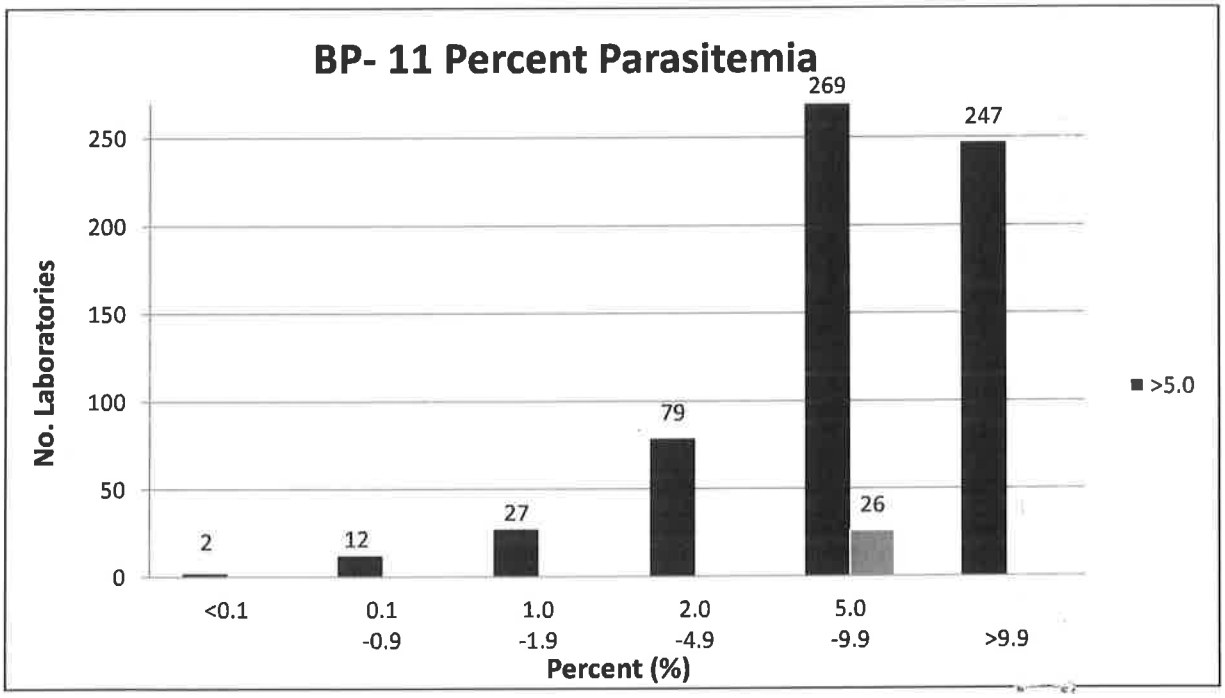
BP-11

Parasite Identification	Referees (50) No.	%	Participants (516) No.	%
Babesia sp.	50	100.0	502	97.3

Parasite Screen	Referees (59) No.	%	Participants (813) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	59	100.0	811	99.8

BP-11

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 October 23, 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-12 Introduction

Thick and thin Giemsa-stained smears were obtained from A 25-year-old Haitian refugee with cyclic fevers. 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.

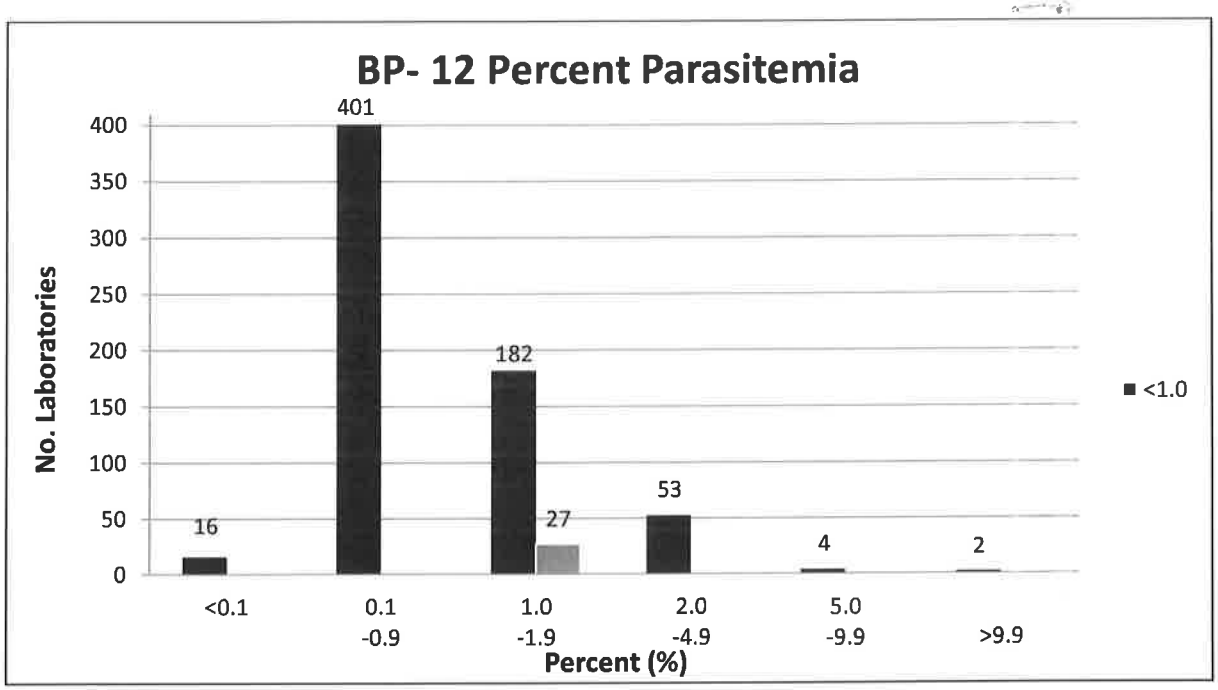
BP-12

Parasite Identification*	Referees (50) No.	%	Participants (521) No.	%
Plasmodium falciparum	34	68.0	330	63.3
Plasmodium sp., NOS would refer or request another specimen, or perform additional molecular testing	11	22.0	81	15.5
Plasmodium sp., not *P. falciparum,* referred for identification	2	4.0	33	6.3
Plasmodium malariae	2	4.0	45	8.6
Plasmodium vivax/ovale, NOS	1	2.0	10	1.9

Parasite Screen	Referees (59) No.	%	Participants (807) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	59	100.0	807	100.0

* Parasite identification graded by referee consensus.

BP-12	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)



Review of the slide demonstrated rings and rare developing trophozoites; thickening of the cytoplasm suggested a possible delay in processing. There were several applique forms but a large number of rings resembling birds-eye forms. While the birds-eye ring form is commonly associated with *P. malariae*, it can be seen with other species too, most-notably *P. falciparum*. Consensus was not reached by participants, however, referees did obtain greater than 80% consensus and the challenge was graded by referee consensus.

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 October 23, 2019.
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 October 23, 2019.
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.
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BP-13 Introduction

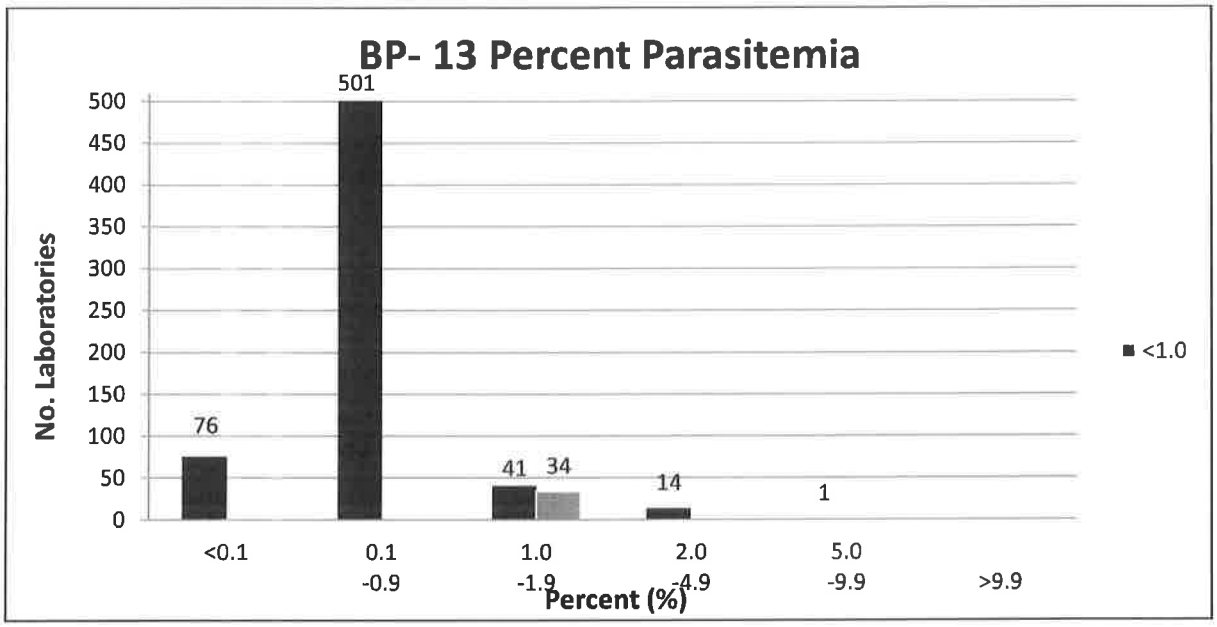
Thick and thin Giemsa-stained smears were obtained from a 44-year-old female with fever returning from visiting family from Ghana. The specimen contained *Plasmodium ovale*. A response of "*Plasmodium ovale*", "*Plasmodium vivax/ovale* NOS", "*Plasmodium* sp., not *P. falciparum* referred for identification", "*Plasmodium* sp., NOS would refer or request another specimen, or perform additional molecular testing", and "*Plasmodium* sp./*Babesia* sp. seen referred for identification" was considered satisfactory.

BP-13

Parasite Identification	Referees (50) No.	%	Participants (516) No.	%
Plasmodium ovale	10	20.0	137	26.5
Plasmodium vivax/ovale, NOS	13	26.0	118	22.8
Plasmodium sp., not *P. falciparum,* referred for identification	19	38.0	131	25.3
Plasmodium sp., NOS would refer or request another specimen, or perform additional molecular testing	3	6.0	36	6.9
Babesia sp.	1	2.0	2	0.4
Plasmodium falciparum	1	2.0	4	0.8
Plasmodium malariae	3	6.0	61	11.8
Plasmodium vivax	1	2.0	34	6.5

Parasite Screen	Referees (59) No.	%	Participants (809) No.	%
Plasmodium sp./*Babesia* sp. seen, referred for identification	58	98.3	804	99.4

BP-13 **If you have identified a *Plasmodium* sp. or *Babesia* sp.: Report percent of infected RBCs seen (number of infected RBCs per 100 red blood cells). (Ungraded)**



Both thick and thin blood films should be prepared when the diagnosis of malaria is suspected. Examination of the thick blood film is considered the gold standard for identification since a larger blood volume can be examined than with the thin film, thus enabling the detection of low levels of parasitemia. In contrast, thin blood films provide the best morphology for species differentiation. Review of this slide showed enlarged round gametocytes and schizonts; mature schizonts had < 15 merozoites. Later stages demonstrated Schüffner's stippling, ruling out *P. malariae*. Elongation and fimbriation was present, albeit rare.

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

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

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

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

Discussion

Refer to BP-12 on page 7 for discussion.

BP-14 Introduction

Thick and thin Giemsa-stained smears were obtained from a 30-year-old laboratorian being monitored after a laboratory accident involving a needle-stick puncture wound. 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-14

Parasite Identification	Referees (50) No.	%	Participants (490) No.	%
Trypanosoma cruzi	50	100.0	484	98.9

Parasite Screen	Referees (59) No.	%	Participants (835) No.	%
Blood flagellate, NOS, referred for identification	57	96.6	741	88.7
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	1	1.7	76	9.1

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.

Molecular diagnosis (PCR) is often employed when morphologic diagnosis is inappropriate. Clinical scenarios that may warrant molecular testing include:

- Person with a bug bite with travel within two months to an endemic country (acute stage after initial exposure).
- Organ transplantation monitoring (after initial serologic testing, below).
- Accidental inoculation (i.e., laboratory accidents)
- Suspect congenital cases

Using PCR to detect *T. cruzi* in blood is only appropriate when the parasitemia is expected to be high, such as in the acute phase of infection or during reactivation following immunosuppression. Molecular testing can be performed at the Centers for Disease Control and Prevention (CDC), where a combination of three different real-time PCR assays are employed, as the different assays have different targets based on the strain of *T. cruzi*.

Diagnosis of chronic *T. cruzi* infection relies on serologic detection of antibodies to this organism. Serologic diagnosis of Chagas disease is challenging. Clinical scenarios that may warrant serologic testing include:

- Screening blood and organ donors
- Symptomatic patients with appropriate travel or exposure history
- Initial transplant recipients with appropriate epidemiologic history or who received donated organs from an individual with appropriate epidemiologic history
- Possible congenital cases.

Unfortunately, no single serologic assay is sensitive and specific enough to be relied upon alone. Therefore, per current recommended guidelines and the CDC, serologic confirmation of chronic *T. cruzi* infection requires reactivity on two tests utilizing two different methodologies and/or two different *T. cruzi* antigen preparations. When results are discordant, a testing by a third assay is recommended to resolve the initial results or, alternatively, repeat testing on a new sample may be required. The CDC performs an ELISA (Chagatest, Wiener Laboratories, Rosario, Argentina) and a Trypomastigote-Excreted Secreted Antigen (TESA) immunoblot (LDT, CDC) for primary testing and an LDT immunofluorescence assay (IFA) for discordant results. A TESA blot can also be obtained commercially (Biomérieux, Rio de Janeiro, Brazil).

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

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2. Bern C, Montgomery SP, Herwaldt BL, et al. Evaluation and treatment of Chagas disease in the United States: A systematic review. *J Am Med Association.* 2007;298:2171-2181.
3. Centers for Disease Control and Prevention, Division of Parasitic Diseases (DPD). Laboratory Identification of Parasites of Public Concern. http://www.cdc.gov/dpdx [accessed October 25, 2019]

BP-15 Introduction

Thick and thin Giemsa-stained smears were obtained from a routine screening of an immigrant from the Philippines with a past history of malaria. A response of "No parasite(s) seen" or "Specimen screened for blood parasites, no organisms seen" was considered satisfactory.

BP-15

Parasite Identification	Referees (50) No.	%	Participants (484) No.	%
No parasite(s) seen	50	100.0	483	99.6

Parasite Screen	Referees (59) No.	%	Participants (843) No.	%
Specimen screened for blood parasites, no organisms seen	58	98.3	837	99.3

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.

COLLEGE of AMERICAN PATHOLOGISTS

Attestation of Participation of Self-Reported Training*

We the participants below have completed the review of the CAP BP-C 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

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