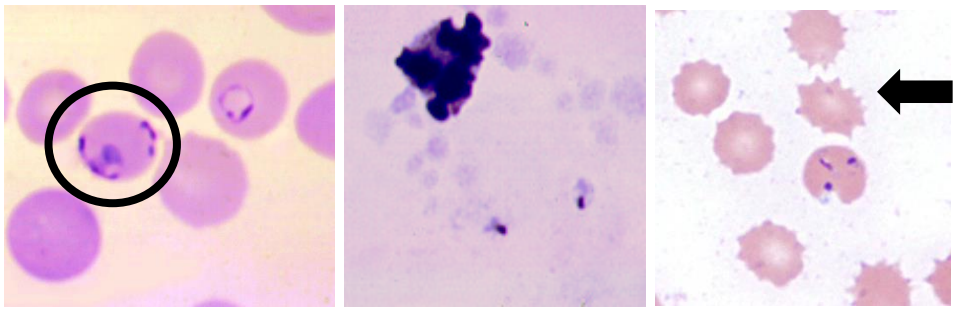
PARASITOLOGY CASE HISTORY #10 (BLOOD PARASITES)
(Lynne S. Garcia)

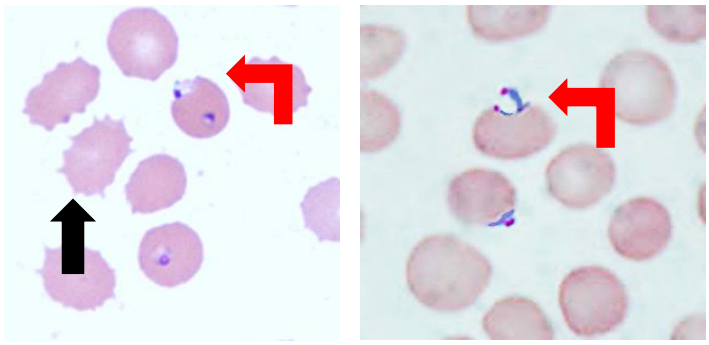
A 12 year old male returned from a visit to West Africa to see his family. He lives in New York. He became ill and was hospitalized with a high fever, vomiting, abdominal pain, and diarrhea. A blood specimen was collected in EDTA for thick and thin blood films and routine hematology. The following images were seen by the hematologist.



What infection most likely matches these images? What key characteristics support your diagnosis?

Answer and Discussion of Blood Parasite Quiz #10

The images presented in this quiz are the following: *Plasmodium falciparum.* Note the multiple rings/RBC, presence of appliqué/accolé forms (circle), and absence of forms other than rings. Additional images can be seen below.



Note the rings that appear to be protruding from the RBC; this can be an important finding to support the diagnosis of *P. falciparum* (red arrows) Some of the images demonstrate RBCs with crenated edges (black arrows); however, this does not represent *P. ovale* with fimbriated edges (sharper points).

Comments on the Patient:

At first glance, the fact that the patient had just returned from his first trip to Africa to visit relatives might suggest a parasitic infection as a possibility. However, if the travel history was not available, there might be no suggestion of malaria. The patient's symptoms were caused by an infection with *Plasmodium falciparum* that he had acquired in Africa. Although the patient had taken prophylaxis for malaria, he had not taken the medication on a regular basis and did not continue to take the medication after returning to the United States. Since he had not been previously exposed to malaria, he was considered immunologically naïve (has no residual antibody from previous exposure). Consequently, he became symptomatic with a relatively low parasitemia. In a patients from endemic areas who have antibody, they will tend to become symptomatic with a much higher parasitemia.

Comments on *Plasmodium falciparum*:

P. falciparum tends to invade all ages of RBCs, and the proportion of infected cells may exceed 50%. Schizogony occurs in the internal organs (spleen, liver, bone marrow, etc.) rather than in the circulating blood. Ischemia caused by the plugging of vessels within these organs by masses of parasitized RBCs will produce various symptoms, depending on the organ involved. It has been suggested that a decrease in the ability of the RBCs to change shape when passing through capillaries or the splenic filter may lead to the plugging of the vessels.

Onset of a *P. falciparum* malaria attack occurs from 8 to 12 days after infection and is preceded by 3 to 4 days of vague symptoms such as aches, pains, headache, fatigue, anorexia, or nausea. The onset is characterized by fever, a more severe headache, and nausea and vomiting, with occasional severe epigastric pain. Diarrhea may also be present. There may be only a feeling of chilliness at the onset of fever. Periodicity of the cycle will not be established during the early stages, and the presumptive diagnosis may be totally unrelated to a possible malaria infection. If the fever does develop a synchronous cycle, it is usually a cycle of somewhat less than 48 h. True relapses from the liver do not occur, and after a year, recrudescences are rare.

Severe or fatal complications of *P. falciparum* malaria can occur at any time during the infection and are related to the plugging of vessels in the internal organs, the symptoms depending on the organ(s) involved. The severity of the complications in a malaria infection may not correlate with the parasitemia seen in the peripheral blood, particularly in *P. falciparum* infections.

Disseminated intravascular coagulation is a rare complication of malaria associated with high parasite burden, pulmonary edema, rapidly developing anemia, and cerebral and renal complications. Vascular endothelial damage from endotoxins and bound parasitized blood cells may lead to clot formation in small vessels. Cerebral malaria is most often seen in *P. falciparum* malaria, although it can occur in the other types as well. If the onset is gradual, the patient may become disoriented or violent or may develop severe headaches and pass into coma. Some patients, even those who exhibit no prior symptoms, may suddenly become comatose. Physical signs of central nervous system involvement are quite variable, and there is no real correlation between the severity of the symptoms and the peripheral blood parasitemia.

Although malaria is no longer endemic within the United States, this infection is considered to be life threatening, and laboratory requests for blood smear examination and organism identification should be treated as ``STAT'' requests. Malaria is usually associated with patients having a history of travel within an area where malaria is endemic, although other routes of infection are well documented. During 2002 (most recent data available), >45% of the malaria cases reported in the United States were caused by *P. falciparum*, the most pathogenic of the four species infecting humans.

Frequently, for a number of different reasons, organism recovery and subsequent identification may be more difficult than the textbooks imply. It is very important that this fact be recognized, particularly when one is dealing with a possibly fatal infection with *P. falciparum*. When requests for malarial smears are received in the laboratory, some patient history information should be made available to the laboratorian. This information should include the following:

1. Where has the patient been and what was the date of return to the United States? (``Where do you live?'')

2. Has malaria ever been diagnosed in the patient before? If so, what species was identified?

3. What medication (prophylaxis or otherwise) has the patient received, and how often? When was the last dose taken?

4. Has the patient ever received a blood transfusion? Is there a possibility of other needle transmission (drug user)?

5. When was the blood specimen drawn, and was the patient symptomatic at the time? Is there any evidence of a fever periodicity? Answers to such questions may help eliminate the possibility of infection with P. falciparum, usually the only species that can rapidly lead to death.

Often the diagnosis of malaria is considered, and a single blood specimen is submitted to the laboratory for examination; however, single films or specimens cannot be relied upon to exclude the diagnosis, especially when partial prophylactic medication or

therapy is used. Partial use of antimalarial agents may be responsible for reducing the numbers of organisms within the peripheral blood, thus leading to a blood smear that contains few organisms, which then reflects a low parasitemia when in fact serious disease is present. Patients with a relapse case or an early primary case may also have few organisms in the blood smear.

Comments on Diagnosis:

It is recommended that both thick and thin blood films be prepared upon admission of the patient, and at least 200 to 300 oil immersion fields should be examined on the thin film before a negative report is issued. Since one set of negative films will not rule out malaria, additional blood specimens should be examined over a 36-h time frame. Although Giemsa stain is recommended for all parasitic blood work, the organisms can also be seen with use of other blood stains such as Wright's stain and the rapid blood stains. Blood collected with use of EDTA anticoagulant is acceptable; however, if the blood remains in the tube for any length of time, true stippling may not be visible within the infected RBCs (*P. vivax*, as an example). Also, when using anticoagulants, it is important to remember that the proper ratio between blood and anticoagulant is necessary for good organism morphology. If the blood stands for >2 hrs prior to blood film preparation, organism distortion is very likely, with the morphology beginning to mimic that seen with *P. malariae*. If the blood stands >6 hrs prior to blood film preparation, organisms will disintegrate.

Malaria is one of the few parasitic infections considered to be immediately life threatening, and a patient with the diagnosis of P. falciparum malaria should be considered a medical emergency because the disease can be rapidly fatal. Any laboratory providing the expertise to identify malarial parasites should do so on a 24-h basis, 7 days/week (STAT orders, collection, processing, examination, reporting).

Plasmodium falciparum **(malignant tertian malaria)**

1. 36-48-hour cycle
2. Tends to infect any cell regardless of age, thus very heavy infection may result
3. All sizes of RBCs
4. No Schüffner's dots (Maurer's dots: may be larger, single dots, bluish)
5. Multiple rings/cell (only young rings, gametocytes, and occasional mature schizonts are seen in peripheral blood)
6. Delicate rings, may have two dots of chromatin/ring, appliqué or accolé forms
7. Crescent-shaped gametocytes

NOTE: Without the appliqué form, Schüffner's dots, multiple rings/cell, and other developing stages, differentiation among the species can be very difficult. It is obvious that the early rings of all four species can mimic one another very easily. **Remember: One set of negative blood films cannot rule out a malaria infection, and this information must be conveyed to the physician.**

Comments on Reporting Parasitemia:

It is important to report the level of parasitemia when blood films are reviewed and found to be positive for malaria parasites. Because of the potential for drug resistance in some of the *Plasmodium* species, particularly *P. falciparum*, it is important that every positive smear be assessed and the parasitemia reported using the exact, same method on followup specimens as on the initial specimen. This allows the parasitemia to be followed after therapy has been initiated. In cases where the patient is hospitalized, monitoring should be performed at 24, 48, and 72 hours after initiating therapy. Generally, the parasitemia will drop very quickly within the first 24 hours; however, in cases of drug resistance, the level may appear to drop, then begin to rise again or the level may not decrease, but actually increase over time.

References:

1. **Garcia, LS,** 2016. *Diagnostic Medical Parasitology*, 6th Ed., ASM Press, Washington, DC.

2. Garcia, LS, 2009. *Practical Guide to Diagnostic Parasitology*, 2nd Ed., ASM Press, Washington, DC

3. **Clinical Laboratory Standards Institute/National Committee for Clinical Laboratory Standards.** 2000. *Laboratory diagnosis of blood-borne parasitic diseases*. Approved Guideline, M15-A. CLSI/NCCLS, Villanova, PA.