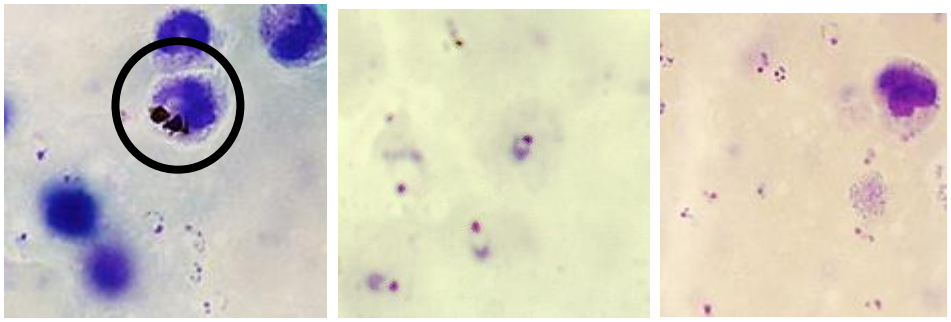
PARASITOLOGY CASE HISTORY #5 (BLOOD PARASITES)
(Lynne S. Garcia)

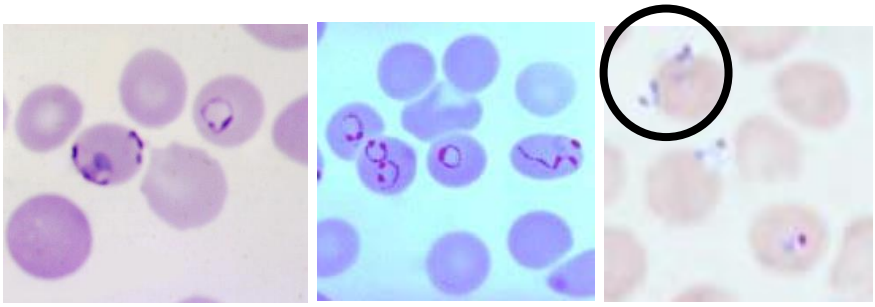
A 47 year old female was seen at a local emergency room with complaints of fevers and chills. She had returned to the United States after living in Africa for several years. A blood smear examination was ordered, and thick and thin blood films were prepared and stained using one of the rapid blood stains. The following images were seen on the thick blood films.



What infection most likely matches these images? What follow-up, if any, would you recommend?

Answer and Discussion of Blood Parasite Quiz #5

The images presented in this quiz are the following: *Plasmodium falciparum.* Below are some images from the thin blood films.



Note the key characteristics: normal size RBCs, appliqué/accolé forms (left image), RBC with two or more rings). In the third frame on the right, note the rings appearing to push outside of the RBC (in circle); this is very typical in *P. falciparum*.

In the original thick film images, note the dark malarial pigment within the WBC (circle). In general the lack of gametocytes and/or schizonts is also

typical for *P. falciparum*; generally rings and developing trophozoites are the only stages seen on stained blood films.

Comments on the Patient:

Because the patient has lived in Africa for some time, she probably has been exposed and has malaria antibodies in her system. Therefore, she has a fairly good parasitemia and was asymptomatic until the number of parasites had already increased. IT IS IMPORTANT TO REMEMBER THAT A PATIENT WITH A VERY LOW PARASITEMIA WILL OFTEN PRESENT TO THE ER WITH SYMPTOMS, BUT A VERY LOW PARASITE COUNT (patient with no residual antibody, immunologically naïve). *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 produces 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 plugging of the vessels.

The onset of a *P. falciparum* malaria attack occurs 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. There may be only a feeling of chilliness at the onset of fever. Periodicity of the cycle is not 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.

Clinical Disease:

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, with 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. Acute lung injury is more likely to occur in patients with very severe, multisystemic *P. falciparum* malaria than in other malaria patients. When patients present with acute lung injury and septic shock, bacterial coinfection should be suspected and treated empirically.

Disseminated intravascular coagulation is a rare complication of malaria; it is associated with a 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 (CNS) involvement are quite variable, and there is no real correlation between the severity of the symptoms and the peripheral blood parasitemia. It has been shown that patients with cerebral malaria were infected with RBC rosette-forming *P. falciparum* and that plasma from these patients generally had no antirosetting activity. A rosette usually consists of a parasitized RBC surrounded by three or more uninfected RBCs. Interaction with adjacent uninfected RBCs in rosettes appears to be mediated by knobs seen on the parasitized RBC. In contrast, *P. falciparum* parasites from patients with mild malaria lacked the rosetting phenotype or had a much lower rosetting rate. Also, antirosetting activity has been detected in the plasma of these patients. These findings strongly support the idea that RBC rosetting contributes to the pathogenesis of cerebral malaria while antirosetting antibodies offer protection against these clinical sequelae.

Key Points - Laboratory Diagnosis:

Although malaria is no longer endemic within the United States, it 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.

Parasite density generally correlates with disease severity, but peripheral parasitemia does not always reflect the number of sequestered organisms. Malaria pigment may serve as a peripheral indicator of parasite biomass, since the pigment can be seen within monocytes and polymorphonuclear leukocytes during light microscopy examination. The presence of pigment has been strongly associated with more severe disease than occurs with uncomplicated cases of malaria. Pigmented neutrophils (polymorphonuclear

leukocytes, monocytes) have been associated with cerebral malaria and with death in children with severe malaria.

Malaria is one of the few parasitic infections considered to be immediately life-threatening, and a patient with the diagnosis of *P. falciparum* or *P. knowlesi* 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 per week.

Frequently, for a number of different reasons, organism recovery and subsequent identification are 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.* Remember that all requests for blood parasite examination are STAT (request, collection, processing, examination, and reporting).

Patient Information. 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?” – this has relevance to “airport” malaria)

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* or *P. knowlesi,* usually the only species that can rapidly lead to death.

1. Blood films should be prepared on admission of the patient (ordering, collection, processing, examination, reporting on a STAT basis). A fever pattern may not be apparent early in the course of the infection

(immunologically naïve patient – travelers); symptoms may be completely random and may mimic any other condition with vague complaints.

2. Both thick and thin blood films should be prepared. At least 200 to 300 oil immersion fields (X 1,000) on both thick and thin films should be examined before the specimen is considered negative.

3. Wright's, Wright-Giemsa, Giemsa, or a rapid stain can be used. The majority of the original organism descriptions were based on Giemsa stain. However, if the white blood cells appear to be well stained, any blood parasites present will also be well stained. The WBCs on the patient smear serve as the QC organism; there is no need to use a *Plasmodium*-positive slide for QC.

4. Malarial parasites may be missed with the use of automated differential instruments. Even with technologist review of the smears, a light parasitemia is very likely to be missed.

5. The number of oil immersion fields examined may have to be increased if the patient has had any prophylactic medication during the past 48 h (the number of infected cells may be decreased on the blood films).

6. *One negative set of blood smears does not rule out malaria. Quantitate organisms from every positive blood specimen.* The same method for calculating parasitemia should be used for each subsequent positive blood specimen.

7. In spite of new technology, serial thick-film parasite counts are a simple, cheap, rapid, and reliable method for identifying patients at high risk of recrudescence due to drug resistance and treatment failure.

8. If you are using any of the alternative methods, make sure you thoroughly understand the pros and cons of each compared with the thick and thin blood film methods.

It is recommended that both thick and thin blood films be prepared on admission of the patient (DO NOT WAIT FOR AN ANTICIPATED FEVER SPIKE, BUT DRAW IMMEDIATELY), and at least 300 oil immersion fields should be examined on both films 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 if

other blood stains, such as Wright's stain or some of the rapid stains, are used. Blood collected with the 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. Heparin can also be used, but EDTA is preferred.

Accurate species diagnosis is essential for good patient management, since identification to the species level may determine which drug or combination of drugs will be indicated. Some patients with *P. falciparum* infections may not yet have the crescent-shaped gametocytes in the blood (may take approximately two weeks). Low parasitemias with the delicate ring forms may be missed; consequently, oil immersion examination at ×1,000 is mandatory.

Malarial parasites may be missed with the use of automated differential instruments. Even with technologist review of the smears, a light parasitemia is very likely to be missed.

The number of oil immersion fields examined may have to be increased if the patient has had any prophylactic medication during the past 48 h (the number of infected cells may be decreased on the blood films).

One negative set of blood smears does not rule out malaria. Quantitate organisms from every positive blood specimen. The same method for calculating parasitemia should be used for each subsequent positive blood specimen.

Epidemiology and Prevention:

Malaria is primarily a rural disease and is transmitted by the female anopheline mosquito. There are great variations in vector susceptibility to infection with the parasite, with many variations being related to differences in parasite strain. Even when the vector is present in an area, an average number of bites per person per day must be sustained or the infection gradually dies out. This critical level can be influenced by a number of factors, including the vector preference for human blood and habitation and the duration of infection in a specific area. Once an area is clear of the infection, there may also be a drop in population immunity, a situation that may lead to a severe epidemic if the infection is reintroduced into the population.

Resistance to *P. falciparum* is also seen in glucose-6-phosphate dehydrogenase-deficient cells. Partial immunity to malarial infection is seen in areas where malaria is endemic when HbC, HbE, β-thalassemia, and pyruvate kinase deficiencies exist.

Report Comments

Report comments can be extremely helpful in conveying information to the physician. Depending on the results of diagnostic testing, the following information can lead to improved patient care and clinical outcomes. The report is provided with the comment following.

1. No Parasites Seen: The submission of a single blood specimen will not rule out malaria; submit additional bloods every 4-6 hrs for 3 days if malaria remains a consideration.

Interpretation/Discussion: It is important to make sure the physician knows that examination of a single blood specimen will not rule out malaria.

2. *Plasmodium* spp. seen: Unable to rule out *Plasmodium falciparum* or *Plasmodium knowlesi*

Interpretation/Discussion: Since *P. falciparum* and *P. knowlesi* cause the most serious illness, it is important to let the physician know these species have NOT been ruled out.

3. *Plasmodium* spp., possible mixed infection: Unable to rule out *Plasmodium falciparum* or *Plasmodium knowlesi*.

Interpretation/Discussion: Since *P. falciparum* and *P. knowlesi* cause the most serious illness, it is important to let the physician know these species have NOT been ruled out.

4. *Plasmodium malariae*: Unable to rule out *Plasmodium knowlesi*

Interpretation/Discussion: If the patient has traveled to the endemic area for *P. knowlesi*, it may be impossible to differentiate between *P. malariae* (band forms) and *P. knowlesi*.

5. *Plasmodium falciparum*: Unable to rule out *Plasmodium knowlesi*

Interpretation/Discussion: If the patient has traveled to the endemic area for *P. knowlesi*, it may be impossible to differentiate between *P. falciparum* (ring forms) and *P. knowlesi*

6. Negative for parasites using automated hematology analyzer: Automated hematology analyzers will not detect low malaria parasitemias seen in immunologically naïve patients (travelers)

Interpretation/Discussion: In patients who have never been exposed to malaria (immunologically naïve), they will become symptomatic with very low parasitemias that will not be detected using automation (0.001 to 0.0001%)

7. Negative for malaria using the BinaxNOW rapid test: This result does not rule out the possibility of a malaria infection. <u>If the rapid test is negative, blood should be submitted for immediate STAT thick and thin blood film preparation, examination, and reporting.</u>

Interpretation/Discussion: The maximum sensitivity of this rapid test occurs at 0.1% parasitemia. Patients (immunologically naïve travelers) may present to the emergency room or clinic with a parasitemia much lower than 0.1%, leading to a false negative report. Also, this rapid test is not designed to identify *P. malariae, P. ovale,* and *P. knowlesi;* the results are most clinically relevant for *P. falciparum* and *P. vivax*. The BinaxNOW is FDA approved for use within the United States.

References:

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

2. **Spudick, J. M., L. S. Garcia, D. M. Graham, and D. A. Haake.** 2005. Diagnostic and therapeutic pitfalls associated with primaquine-tolerant *Plasmodium vivax. J. Clin. Microbiol.* **43**:978–981.

3. **Lou, J., R. Lucas, and G. E. Grau.** 2001. Pathogenesis of cerebral malaria: recent experimental data and possible applications for humans. *Clin. Microbiol. Rev*. 14:810–820.

4. **Calderaro, A, G Piccolo, C Gorrini, S Rossi, S Montecchini, ML Dell'Anna, F De Conto, MC Medici, C Chezzi, MC Arcangeletti.** 2013.

Accurate identification of the six human *Plasmodium* spp. causing imported malaria, including *Plasmodium ovale wallikeri* and *Plasmodium knowlesi.* *Malar J* **12**:321.

5. **Garcia, L. S., R. Y. Shimizu, and D. A. Bruckner**. 1986. Blood parasites: problems in diagnosis using automated differential instrumentation. Diagn. Microbiol. Infect. Dis. 4:173–176.

6. **Garcia, LS**, 2010. Malaria. Clin Lab Med 30:93-129.