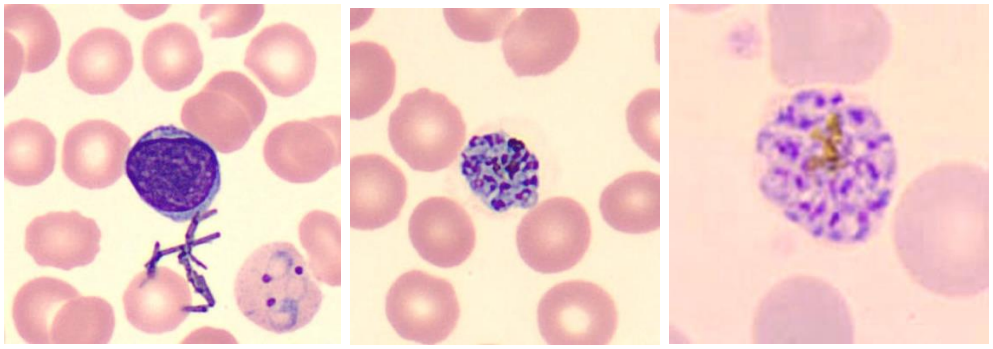
PARASITOLOGY CASE HISTORY #8 (BLOOD PARASITES)
(Lynne S. Garcia)

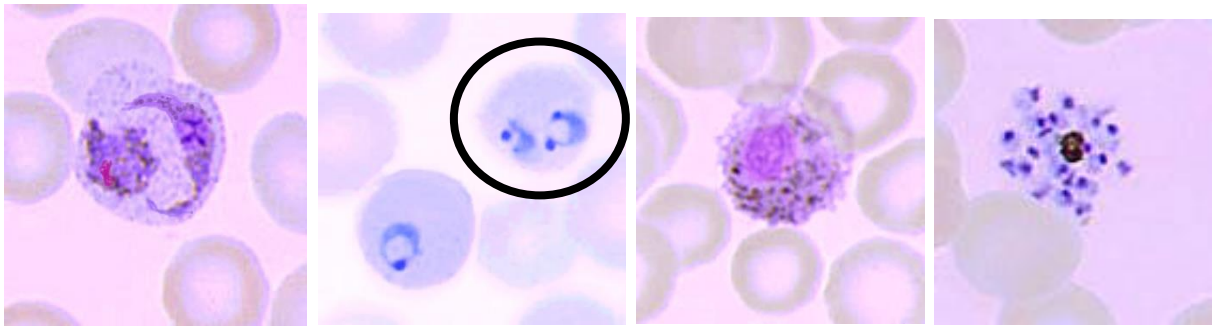
A 2 year old female was seen at a local emergency room with 10 days of fevers and chills. She had moved with her family to the United States from Afghanistan approximately three months before. Routine blood work was ordered, including thick and thin blood films that were stained with Wright-Giemsa stain. The following images were seen on routine microscopic examination of the thin blood film. Case courtesy of Texas Children's Hospital, Houston, TX and CDC.



What infection most likely matches these images?

Answer and Discussion of Blood Parasite Quiz #8

The images presented in this quiz are the following: *Plasmodium vivax.* Note the bacterial contaminants in the left image seen above. Below are some additional representative images with *P. vivax.*



Note the key characteristics: enlarged infected RBCs, RBC with two or more rings – not limited to *P. falciparum)*, mature macrogametocyte, and (far right) mature schizont containing approximately >16 merozoites.

Comments on the Patient:

The patient acquired the infection while living in Afghanistan. It is unclear whether this is an initial infection of a relapse. Of the five species that infect humans, *P. vivax* and *P. falciparum* account for 95% of infections. Some estimates indicate that *P. vivax* may account for 80% of the infections. This species also has the widest distribution, extending throughout the tropics, subtropics, and temperate zones. *P. falciparum* is generally confined to the tropics, *P. malariae* is sporadically distributed, and *P. ovale* is confined mainly to central West Africa and some South Pacific islands.

Clinical Disease:

The primary clinical attack usually occurs 7 to 10 days after infection, although there are strain differences, with a much longer incubation period being possible. In some patients, symptoms such as headache, photophobia, muscle aches, anorexia, nausea, and sometimes vomiting occur before organisms can be detected in the bloodstream. In other patients, the parasites can be found in the bloodstream several days before symptoms appear.

Clinical symptoms of malaria include anemia, splenomegaly, and the classic paroxysm, with its cold stage, fever, and sweats. Although the febrile paroxysms strongly suggest infection, many patients who are seen in medical facilities, particularly in the early stages of the infection, do not exhibit the typical fever pattern. They may have fever or several small, random peaks each day. Since the symptoms associated with malaria are so nonspecific, the diagnosis should be considered in any symptomatic patient with a history of travel to an area where malaria is endemic. During the primary infection in a nonimmune host, the early fever episodes can affect density-dependent regulation of the parasite population, maintaining cycles of parasitemia and promoting synchronous parasite growth. The typical paroxysm begins with the cold stage and rigors lasting 1 to 2 h. During the next few hours, the patient spikes a high fever and feels very hot, and the skin is warm and dry. The last several hours are characterized by marked sweating and a subsequent drop in body temperature to normal or subnormal.

During the first few days, the patient may not exhibit a typical paroxysm pattern but, rather, may have a steady low-grade fever or an irregular remittent fever pattern. Once the typical paroxysms begin, after an irregular periodicity, a regular 48-h cycle is established. An untreated primary attack may last from 3 weeks to 2 months or longer. The paroxysms become less severe and more irregular in frequency and then stop altogether. In 50% of patients, relapses occur after weeks, months, or up to 5 years (or more).

Severe complications are rare in *P. vivax* infections, although coma and sudden death or other symptoms of cerebral involvement have been reported. These patients can exhibit cerebral malaria, renal failure, circulatory collapse, severe anemia, hemoglobinuria, abnormal bleeding, acute respiratory distress syndrome, and jaundice. Studies have confirmed that these were not mixed infections with *P. falciparum* but single-species infections with *P. vivax.* Recent data demonstrate that the infection comes with a significant burden of morbidity and associated mortality.

Since *P. vivax* infects only the reticulocytes, the parasitemia is usually limited to around 2 to 4% of the available RBCs. Splenomegaly occurs during the first few weeks of infection, and the spleen progresses from being soft and palpable to hard, with continued enlargement during a chronic infection. If the infection is treated during the early phases, the spleen returns to its normal size.

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; if the rapid test is negative, then immediately perform thick and thin blood film examinations before reporting the specimen as negative.

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 $\times$1,000 is mandatory.

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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.

Duffy antigen-negative RBCs lack surface receptors for *P. vivax* invasion. Many West Africans and some American blacks are Duffy antigen negative,

which may explain the low incidence of *P. vivax* in West Africa. In other areas of Africa, *P. vivax* is much more prevalent.

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. If the rapid test is negative, blood should be submitted for immediate STAT thick and thin blood film preparation, examination, and reporting.

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:

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