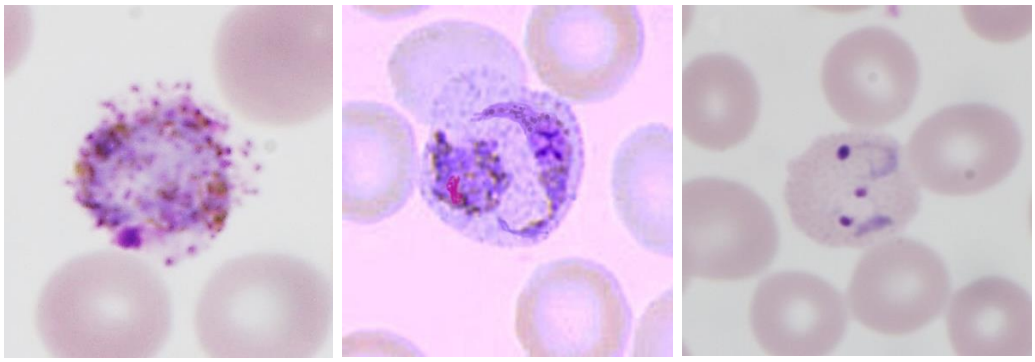
PARASITOLOGY CASE HISTORY #1 (BLOOD PARASITES)
(Lynne S. Garcia)

A 47 year old female was seen at a local emergency room with complaints of fevers and chills. She had been traveling in Thailand for approximately three weeks. When she traveled abroad in the past, she had taken malaria prophylaxis from the travel clinic. However, she did not visit the clinic prior to this trip, her most recent trip abroad.

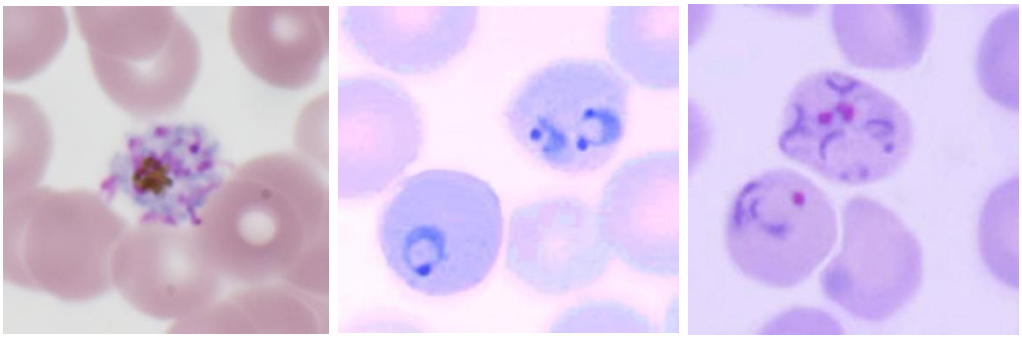
A blood smear examination was ordered, and thick and thin blood films were prepared and stained using one of the rapid blood stains. The following images were seen on the blood films.



What infection most likely matches these images? Based on the images seen above why might there be some confusion regarding the species?

Answer and Discussion of Blood Parasite Quiz #1

The images presented in this quiz are the following: *Plasmodium vivax*



Note the key characteristics: enlarged RBCs, mature schizont with ~18 merozoites, RBC with two rings (this may be confused with *Plasmodium*

falciparum, but is also seen in *P. vivax)*. In the third frame, there are no visible Schüffner's dots (occurs if blood has been standing in EDTA too long prior to smear preparation).

Comments on the Patient:

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.

We usually associate malaria with patients having a history of travel within an area where malaria is endemic. However, other situations that may result in infection involve the receipt of blood transfusions, use of hypodermic needles contaminated by prior use (as with, for example, drug addicts), possibly congenital infection, and transmission within the United States by indigenous mosquitoes that acquired the parasites from imported infections.

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.

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.

Leukopenia is usually present; however, leukocytosis may be present during the febrile episodes. Concentrations of total plasma proteins are unchanged, although the albumin level may be low and the globulin fraction may be elevated. The increase in the concentration of gamma globulins is caused by the development of antibodies. The level of potassium in serum may also be increased as a result of RBC lysis.

Key Points - Laboratory Diagnosis:

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?

It is recommended that both thick and thin blood films be prepared on admission of the patient, and at least 200 to 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. Finger stick blood is recommended, particularly when the volume of blood required is minimal (i.e., when no other hematologic procedures have been ordered). The blood should be free flowing when taken for smear preparation and should not be contaminated with alcohol used to clean the finger prior to the stick.

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. Low parasitemias with the delicate ring forms may be missed; consequently, oil immersion examination at $\times$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.

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.

Final Comments:

References:

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