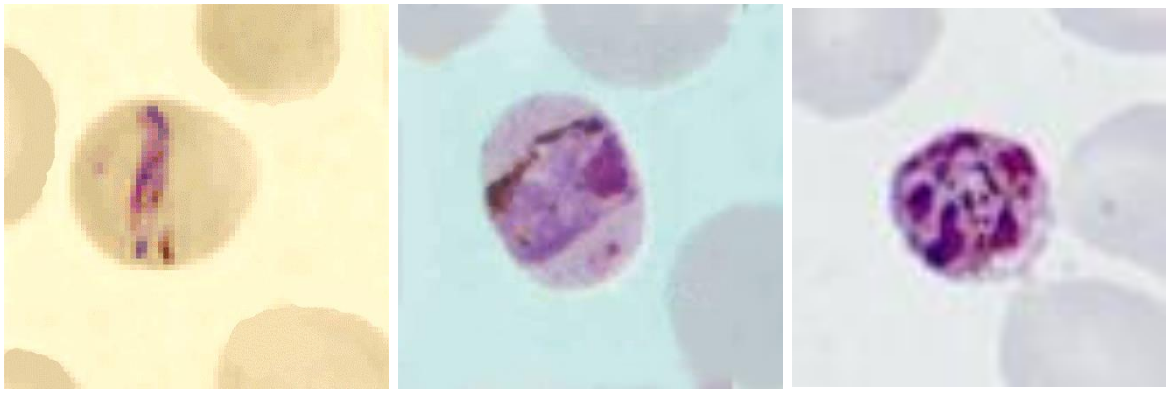
PARASITOLOGY CASE HISTORY #11 (BLOOD PARASITES)
(Lynne S. Garcia)

A 39-year old male traveler developed fever and thrombocytopenia after returning from a trip to the Philippines. The parasitemia was 10,000 parasites per microliter of blood (0.2%). While the rapid antigen test was negative, routine slide examination using microscopy revealed the following images:



Although the presumptive diagnosis was *Plasmodium malariae*, molecular testing revealed a different identification.

Please identify the organism. What is there about the patient history that might suggest an alternative diagnosis?

Discussion of Blood Parasite Quiz #11

The images presented in Diagnostic Blood Parasite Quiz #11 are the following:

These images show examples of *Plasmodium knowlesi* malaria stages (left to right: band form, band form, developing schizont). **It is important to remember that *P. knowlesi* infection should be suspected in symptomatic travelers returning from South East Asia.** Since the rapid tests were not originally produced including the detection of *P. knowlesi*, and they are also quite insensitive for *P. malariae*, confirmation of the species will often require molecular tests.

Life cycle: Preerythrocytic Cycle. The vector for malaria is the female anopheline mosquito. When the vector takes a blood meal, sporozoites contained in the salivary glands of the mosquito are discharged into the puncture wound. Within an hour, these infective stages are carried via the

blood to the liver, where they penetrate hepatocytes and begin to grow, thus initiating the pre-erythrocytic or primary exoerythrocytic cycle. Detailed study of sporozoite entry into the hepatocytes indicates that the process involves parasite-encoded surface proteins and host molecules. The sporozoites become round or oval and begin dividing repeatedly. This schizogony results in large numbers of exoerythrocytic merozoites.

Erythrocytic Cycle. Once these merozoites leave the liver, they invade the red blood cells (RBCs), thus initiating the erythrocytic cycle). A secondary or dormant schizogony may occur in *P. vivax* and *P. ovale* organisms, which remain quiescent in the liver until a later time. These resting stages have been termed hypnozoites. Delayed schizogony does not occur in *P. falciparum* and probably does not occur in *P. malariae* or *P. knowlesi.*

Recrudescence. The situation in which the RBC infection is not eliminated by the immune system or by therapy and the number in the RBCs begins to increase again with subsequent clinical symptoms is called a recrudescence. All species may cause a recrudescence.

True Relapse. The situation in which the erythrocytic infection is eliminated and a relapse occurs later because of a new invasion of the RBCs from liver merozoites, called a recurrence or true relapse, theoretically occurs only in *P. vivax* and *P. ovale* infections

***Plasmodium knowlesi*.**

In addition to the Kapit Division of Sarawak in Malaysian Borneo, there have also been reports of locally acquired *P. knowlesi* infections from Southern Thailand, the Myanmar-China border, the Philippines, and Singapore, indicating that transmission occurs in many Southeast Asian countries. *P. knowlesi* is primarily a chronic infection of the long-tailed *(Macaca fascicularis)* and pig-tailed *(Macaca nemestrina)* macaques. **THE EARLY STAGES ARE EASILY CONFUSED WITH *P. FALCIPARUM*, WHILE THE LATER STAGES MIMIC *PLASMODIUM MALARIAE* ON BLOOD FILM MICROSCOPY IN CASES OF HUMAN INFECTION.** The erythrocyte invasion by *P. knowlesi* is not limited to young or old cells, which allows high parasitemia, and development of the parasite in erythrocytes is asynchronous.

P. knowlesi is relatively closely related with *P. vivax*; therefore, it is not surprising that infections with these 2 species share some common features, such as occasional severity and marked thrombocytopenia. However, disease caused by *P. knowlesi* is somewhat more severe than disease caused by *P. vivax*, although also vivax malaria can be severe in a significant number of

cases, at least in certain regions (severe malaria in ~3%, compared with 7% in individuals with *P. knowlesi* malaria).

In Malaysia, the patients with *P. knowlesi* infection typically present with a febrile illness with daily fever and chills. Other frequent symptoms included headache, rigors, malaise, myalgia, abdominal pain, breathlessness, and productive cough. Tachypnea, pyrexia, and tachycardia were common clinical signs. Although the patients had thrombocytopenia at hospital admission or on the following day, none had clinical coagulopathy. At hospital admission, only a few of the patients had anemia, whereas mild hepatic dysfunction was relatively common. The great majority of patients (94%) experienced no complications, and the infection responded well to chloroquine and primaquine treatment. In patients with severe infections, the most frequent complication was respiratory distress.

P. knowlesi is unique amongst the primate and human malarias in that it has a 24-h erythrocytic cycle, which tends to accelerate the development of complications. Information on the characteristics of knowlesi malaria in humans indicates the levels of parasitemia vary with a wide range among different patients. The *P. knowlesi* parasitemia at hospital admission tends to be strongly and independently associated with renal dysfunction; renal failure is seen despite interventions. Unlike serious cases of *P. falciparum*, neurological symptoms with *P. knowlesi* are rare, and no cases of cerebral malaria have been reported. However, autopsy findings in a fatal case of *P. knowlesi* malaria indicate that *P. knowlesi*–infected red cells can sequester in capillaries of the brain, heart, and kidneys. In some of the fatal cases, a high parasitemia was seen (15%, >10%, and >10 parasites per high-power microscope field).

Since *P. knowlesi* malaria can progress rapidly to severe disease, these presumptive cases should be treated like *P. falciparum* malaria if the species identification is based on microscopic examination alone or if coinfection with *P. falciparum* cannot be excluded with certainty using PCR. It is important to remember that diagnostic results should include a comment: "Unable to rule out *P. falciparum* or *P. knowlesi*." This information alerts the physician to the possibility of severe disease and possible fatal outcomes. This approach is particularly important if the microscopic examination of blood films suggests *P. malariae*, but the patient has severe disease, a high parasitemia (> 0.1%) or a recent history of visiting woods or their vicinity in Southeast Asia. Unfortunately, neither the pLDH- nor aldolase-based rapid tests demonstrate sufficiently high overall sensitivity for *P. knowlesi*. More sensitive rapids are needed in regions of *P. knowlesi* endemicity; new test development is currently underway.

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
.
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.*

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.

Conventional Microscopy. Often, when the diagnosis of malaria is considered, only a single blood specimen is submitted to the laboratory for examination; however, single films or specimens cannot be relied on 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 in 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.

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 (60, 61). 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, 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.

KEY POINTS – LABORATORY DIAGNOSIS

Malaria

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; see chapter 7 for additional information on diagnostic methods for blood parasites.

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*

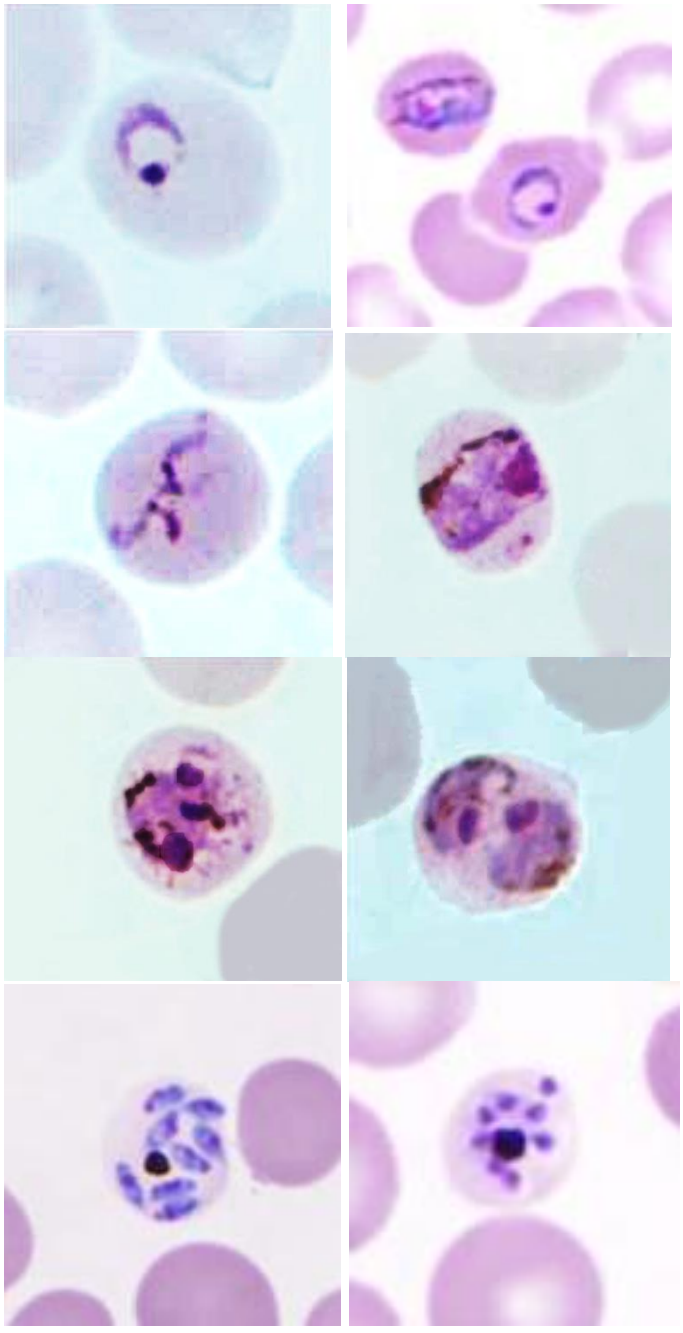
6. Negative for parasites using automated hematology analyzer: Automated hematology analyzers will not detect low malaria parasitemias seen in immunologically naïve patients (travelers)

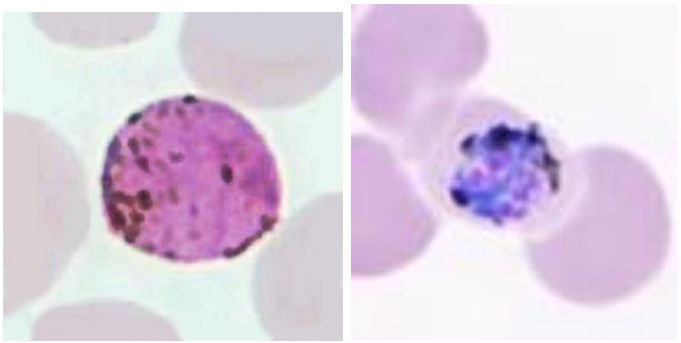
Interpretation/Discussion: In patients who have never been exposed to malaria (immunologically naïve), they will become symptomatic with very low parasitemias that will not be detected using automation (0.001 to 0.0001%)

7. Negative for malaria using the BinaxNOW rapid test: This result does not rule out the possibility of a malaria infection. Blood should be submitted for STAT thick and thin blood film preparation, examination, and reporting.

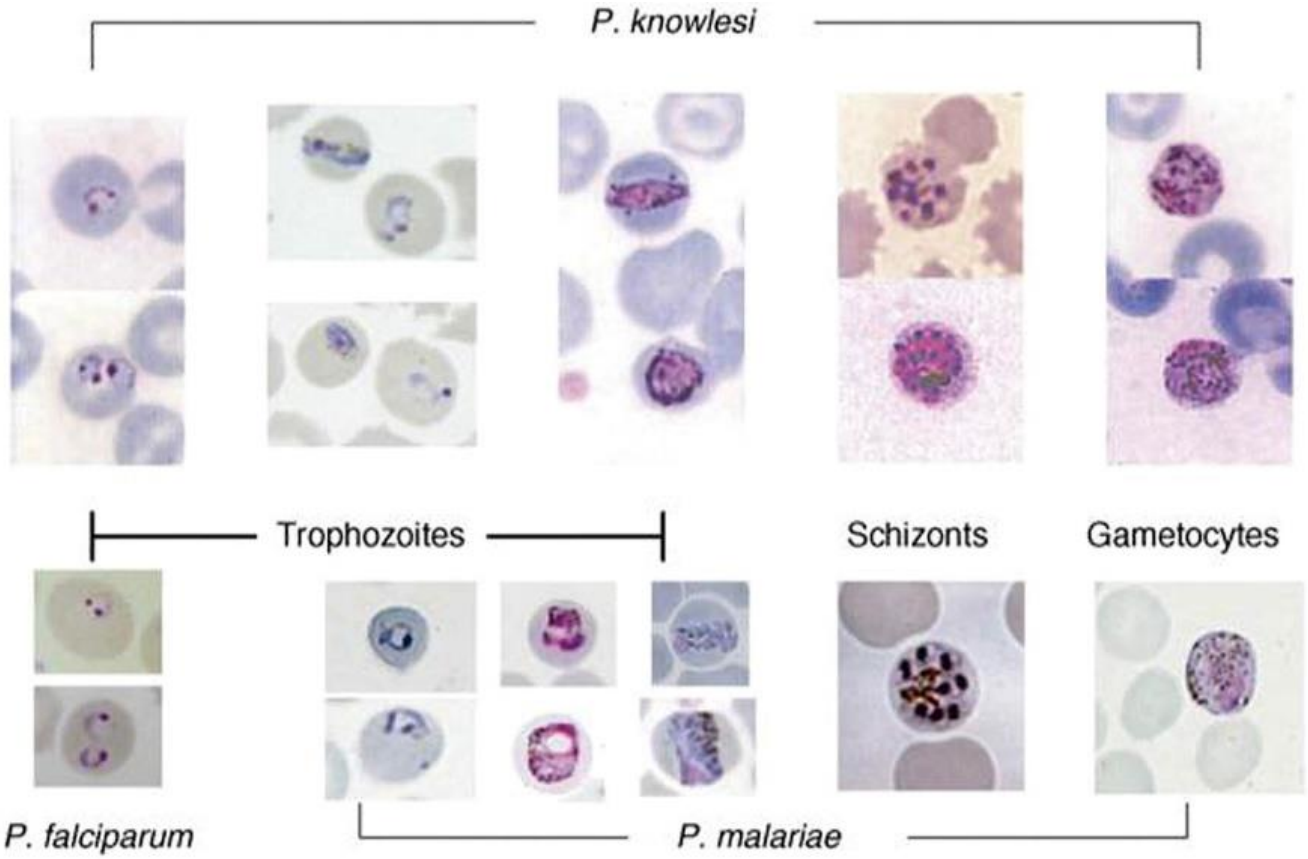
Interpretation/Discussion: The maximum sensitivity of this rapid test occurs at 0.1% parasitemia. Patients (immunologically naïve travelers) may present to the emergency room or clinic with a parasitemia much lower than 0.1%, leading to a false negative report. Also, this rapid test is not designed to 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.

Images:





Plasmodium knowlesi. . (Top three rows) **<u>Developing trophozoites (note the band forms can mimic *P. malariae*; ring forms can mimic *P. falciparum*);</u>** (Fourth row) Mature schizonts; (Bottom row) Gametocytes (Courtesy of the CDC Public Health Image Library).



Geographic region

Macaques

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