Review Article

A review of one year malaria blood film data from a hospital in Yei, South Sudan

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ABSTRACT

Although Rapid Diagnostic Tests (RDTs) are utilized more frequently today, blood films are still the gold standard for diagnosing malaria. This is an observational study, looking at the experience of His House of Hope and Faith Hospital (HHHF) in Yei, South Sudan, in one calendar year (January to December in 2023) tracking *Plasmodium falciparum (PF)* and other plasmodium species. We report some simultaneous data using RDT for malaria and data regarding co-infection with *Salmonella typhi* (Typhoid fever).

Keywords: malaria, blood film, Rapid Diagnostic Test, South Sudan

Introduction

Malaria is one of the most common diagnoses in sub-Saharan Africa, particularly in South Sudan. Data from WHO suggests there are 100-300 cases/1000 people per year,[1] equivalent to one case in 3-4 people. This means that South Sudan is one of the countries most heavily affected globally, but that may be an underestimation. Over 90% of the Hospital staff are often infected every year, many of them multiple times (personal observation). This is an observational report of the number of malaria-positive blood films performed in a calendar year at His House of Hope and Faith Hospital (HHHF), Yei, South Sudan.

Method

Thick and thin blood films were performed by the HHHF laboratory staff under the supervision of KO and AJ when requested by the hospital clinicians. A blood slide is prepared from a finger prick with thick and thin smears on the same slide as follows:

1. The ring finger is cleaned with an alcohol swab, dried, and pricked using a blood lancet.
2. A small drop of blood, approximately six microlitres, is applied to a clean glass slide for the thick film and similarly, approximately two microlitres for the thin film, which is spread using another clean glass slide positioned at 30 to 45 degrees to allow the blood to run along the plane. Pushing gently, the thin smear is created and should be V-shaped with feathered ends. Using the same spreader, six microlitres of blood are gently mixed in a circular motion,

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starting from inside, going outwards, and then to the centre. A good thick smear is round.

3. The smear is air-dried for five minutes. The thin smear is fixed using absolute methanol.

The thick and thin smears are then heat-fixed for at least 2 to 3 minutes. The slides are allowed to cool. They are stained using 10% Giemsa stain solution, rinsed in water, and dried for microscopic examination. At least 200 microscope views under oil immersion are examined before a slide is declared negative for malarial parasites. Both asexual and sexual parasites are counted as positive. We also check for different malarial species. The parasites are designated either *Plasmodium falciparum* (PF) or "other" Plasmodium species for this reporting.

Rapid Diagnostic Tests (RDTs) for malaria are performed either by the laboratory or the clinician using Abbott RDT for PF and other plasmodium species. KO or AJ performed an RDT test for *Salmonella typhi* IgM from Parl Care Accurate.

Results

As indicated in Figure 1, the number of blood films for malaria requested by HHHF hospital clinicians varied throughout the year. The lowest number of tests ordered was in December and was 49% of the number requested in June. The number of positive slides also showed some variation - with those positive for PF in December being 49% of the highest number in July. Over 84% of the blood films were positive for either PF or another plasmodium species from May to August. 75% of the blood films were

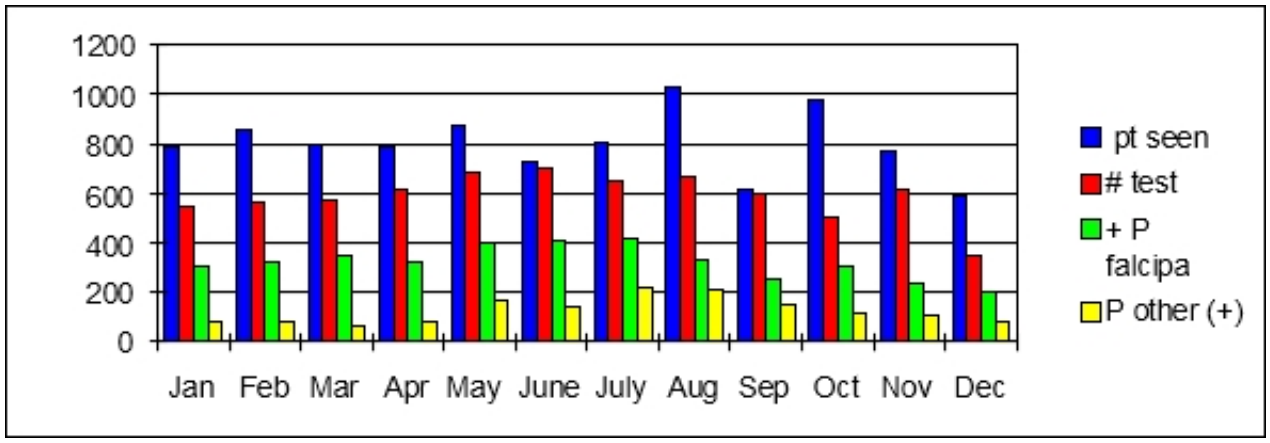

Figure 1. Blue columns represent the total number of patients seen that month. The red columns are the number of patients tested for malaria using blood film. The green columns are the number of blood films (+) for PF. The yellow columns are the number of blood films (+) for other plasmodia species.

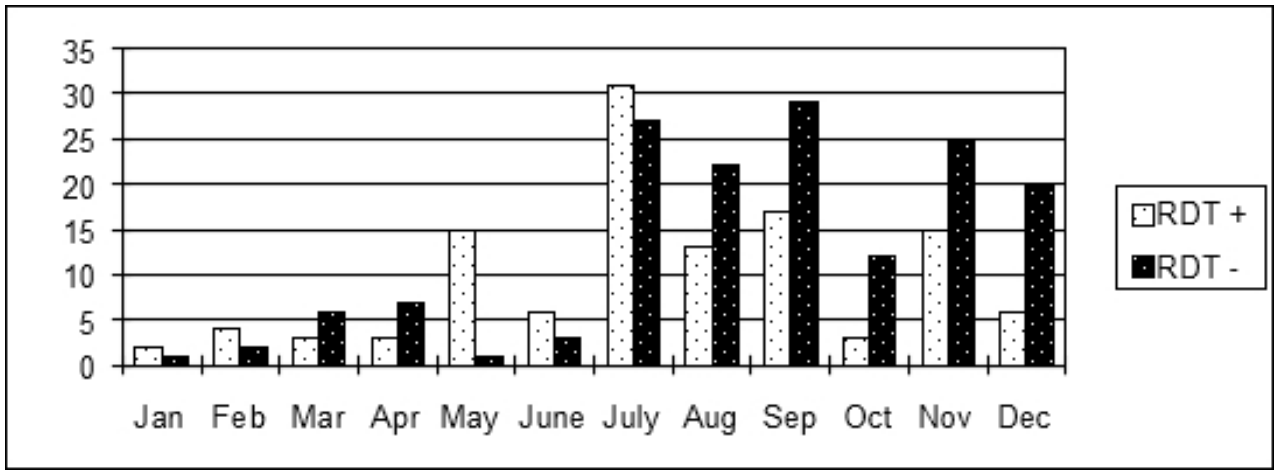

Figure 2. The white columns are the number of Rapid Diagnostic Tests for malaria that were (+). The black columns are the negative tests

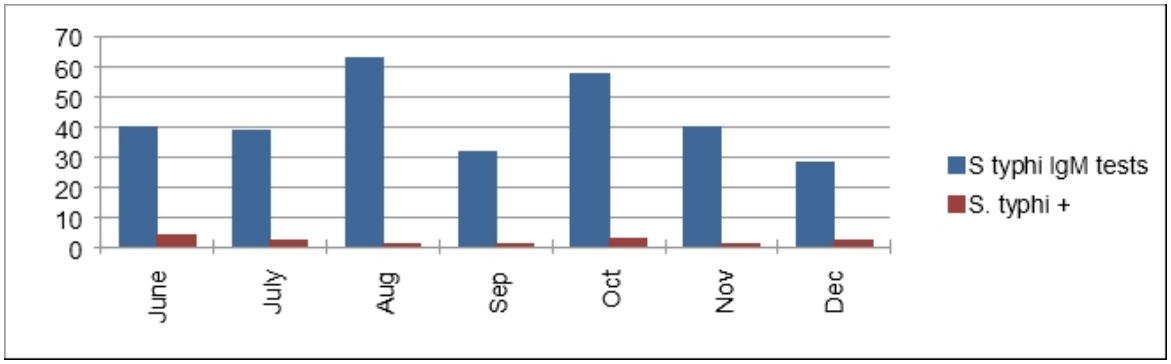

Figure 3. The blue column represents the total number of tests for S. typhi IgM. The red columns represent the number of (+) tests.

positive for PF or another species. RDTs were performed much less often than blood films and were positive at a lower percentage. From May to August, 60% of the RDTs were positive compared to 84% of blood films. Only 44% of the RDTs were positive for the year, whereas 75% of the blood films were positive (see Figure 2).

Three hundred *Salmonella typhi* (S typhi) IgM tests were performed over a seven-month period on patients suspected of having typhoid fever, but only 14 (4.7%) were positive. See Figure 3. Most of these tests were performed on patients who were also tested for malaria.

Discussion

Malaria was the most common diagnosis in our outpatient clinic during the calendar year 2023 and reflects the experience in most of South Sudan. However, Yei is in the southernmost part of South Sudan; hence, the seasonal patterns observed there are not the same as in the remainder of the country. As expected, *Plasmodium falciparum* (PF) was the most common malaria parasite, but other species were also found in a significant quantity (see Figure 1), as has been reported from Zambia.[1] There was a rise in cases seen during the rainy season, with the highest numbers of malaria confirmations from May to August. Though the number of tests ordered in August and September were similar (95%), the positive number in September was only 57% of those in August. There was a slight decline from September to December compared to the earlier months.

One interesting observation was the very low IgM-positive tests for *Salmonella typhi* obtained during the latter half of this period. From June to December, the number of malaria cases was high (2,253), but the number of confirmed cases of *S. typhi* was low (14). Many people in South Sudan speak of "malaria-typhoid" as though the two diseases have a special relationship, a synergistic effect, or that one predisposes to the other, as HIV does for TB. One study that suggested a strong relationship between the two diseases relied on the Widal test.[3] Studies examining more specific tests for *S. typhi* have shown modest to no association.[4,5] Using blood film or RDT, malaria is a common diagnosis in South Sudan and sub-Saharan Africa, but typhoid fever is more of an Asian disease. In confirmed cases of typhoid fever using specific data (cultures or IgM antibodies), 75-80% are in Asia.[4]

Our data suggest that if there is an association between the two diseases, it is small and probably coincidental. IgM antibodies reflect current or recent infection and are the only specific antibody test for an acute infection for any pathogen. IgG-positive antibodies reflect infection in the past. The Widal test is non-specific, even with high titres. Malaria is common as expected and will often occur with other diseases, but our data suggests that there is no special relationship between malaria and typhoid fever.[4,5]

The studies mentioned above[4,5] have also failed to show synergy between the two diseases when specific tests for *S. typhi* are employed. We suggest this presumed relationship is probably caused by the over-reliance in South Sudan on the Widal test, a non-specific test that at best helps support the diagnosis when the clinical picture strongly points to typhoid fever but should not be used to make the diagnosis without other evidence. Now that more specific tests for *S. typhi* are available, we suggest the Widal test be abandoned.

It is important to be cautious when drawing conclusions from an observational study. However, our study in Yei shows that, although there may be a "malaria season," there is no time of the year when malaria ceases to be commonly

found, especially near a river. Secondly, although PF is the most common form of malaria in South Sudan, it is not the only malaria species. This may be important to those centres that rely on Rapid Diagnostic Tests that test for PF only. A recent study from Ethiopia, including Sudan and South Sudan, suggests that RDTs may be missing up to 20% of cases because of some evolution of antigens in PF.[6] We do not know if that was the mechanism, but RDTs were less sensitive than blood films in our hands. We suggest that this reliance on RDTs needs to be re-examined. Thirdly, typhoid fever is not a common co-infection with malaria, and the term "malaria-typhoid" needs to be discarded. We suggest that if typhoid is suspected and whether or not there is a concomitant infection with malaria, it should be confirmed by *S. typhi* IgM antibodies, not the Widal test. From the available data, there is no justification for presumptively treating typhoid fever in all patients with malaria.

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