Field evaluation of the QBC technique for rapid diagnosis of vivax malaria

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The QBC (quantitative buffy coat) technique was compared with that of the Giemsa-stained thick blood film (GTF) under field conditions in Junlian and Mingshan counties, Sichuan, China, for rapid diagnosis of vivax malaria. Blood samples were collected from 364 volunteer villagers, and each sample was examined with both the QBC and GTF techniques. For each GTF sample (10 μl of blood), as many as 300 oil-immersion fields were examined; each QBC tube was inspected for up to 5 minutes. The GTF technique resulted in 86 positive blood samples and 278 negative; the QBC technique indicated 89 positive and 275 negative samples. Relative to the results obtained with GTF, the QBC technique had a sensitivity and specificity of 87.2% and 95.0%, respectively; concordance between the tests was 93.1%. The median time-to-positive diagnosis with the QBC technique (1.12 min) was 11% of that with GTF. The distribution of different developmental stages of Plasmodium vivax parasites was also examined in the centrifuged QBC tubes: all stages except schizonts could be found in the lower part of the platelet zone (the interphase between the monocyte and platelet layers), especially ring forms.

Introduction

Although quite a number of immunodiagnostic methods have been introduced, the accurate diagnosis of active malaria infection still depends on microscopic examination of Giemsa-stained thick blood films (GTF). Specific DNA probes for the detection of malarial parasites have been reported (1); however, owing to limitations in their sensitivity and the inconvenience of using radiolabelled reagents, their application in developing countries at present is hardly practicable. None the less, it has been generally acknowledged that the GTF method is tedious and time-consuming in both the preparation and examination phase. Furthermore, when levels of parasitaemia in the examined population are low, a great deal of experience with the technique is needed to find parasites. Recently, some investigators have found that malaria can be diagnosed by direct observation of centrifuged samples of blood (2, 3).

The method, referred to as the quantitative buffy coat (QBC) technique, is easy to perform. Moreover, the QBC technique has the advantage of speed, and its sensitivity and specificity are good when compared with the GTF method.

Previous studies in Ethiopia (2), the Philippines (3), Thailand (4), and Indonesia (5) concerned mainly falciparum malaria or mixed infections of falciparum and vivax malaria. Since the specific gravity of an erythrocyte parasitized by Plasmodium falciparum differs from that of one parasitized by Plasmodium vivax (6), the success rate and speed of diagnosis for the two species may be different. In order to ascertain whether the QBC technique could also be practical for antimalarial activities in Sichuan Province, China, an endemic area where exclusively vivax malaria occurs with low levels of parasitaemia, we carried out a field evaluation of the effectiveness of the QBC technique in western and south-eastern Sichuan during the transmission season (June–August) of 1992 and 1993.

Materials and methods

Study site and subjects

The study was conducted in Sifang, a small village in the central part of Junlian county on the southern border of Sichuan Province, and in Guantian and Pingqiao villages in Mingshan county in the western part of the province.
A total of 407 villagers volunteered and donated blood samples, but only 364 specimens (178 from men and 186 from women; age range, 4 months to 84 years) were of large enough volume to be examined by both the GTF and QBC methods. An additional 218 samples were collected from suspected malaria patients in regional hospitals.

Sample collection and film preparation
Using disposable sterile lancets, 300 µl of blood was collected from each subject by finger- or ear-prick, and drawn into a 1.5-ml centrifuge tube containing 15 µl of anticoagulant. All specimens were delivered to the field laboratory within 4 hours and processed immediately.

GTFs were prepared in the standard way, i.e. 10 µl of blood was spread evenly over an area of 12-mm diameter, air-dried overnight, dehaemoglobinized, fixed, and stained with Giemsa stain. At the same time, thin blood films were made and reserved for later parasite quantification and species identification as necessary.

QBC technique
The QBC blood-parasite detection system (Becton-Dickinson Co., Franklin Lakes, NJ, USA) was used in the investigation. It consists of a battery-powered centrifuge, an ultraviolet (UV) microscope-adapter fibre optic system, a microscope tube holder, and a 20-place tube holder rack. Additionally, QBC malaria diagnosis kits (QBC tubes, floats, closures, and lancets), were contributed by Becton–Dickinson World Wide, Singapore, and were stored at 16–37°C before use.

For the QBC test, 55 µl of blood was drawn into the QBC tube within 4–6 hours after collection. The blood was mixed with the precoated contents by gently rotating and tilting the tube for at least 5 seconds. Tubes were immediately centrifuged in batches of 20 at 12,000 rpm (14000g) for 5 minutes and set up on the rack. Using the UV microscope-adapter fibre optic system, with a 60× oil-immersion objective, each tube was rested in the holder and examined for malarial parasites beginning with the upper part of the red-blood-cell layer and continuing through the buffy coat area down to the platelet layer.

Microscope reading and recording of results
During the microscopic examination of GTFs or QBC tubes, we used a fixed stopping point as follows: as many as 300 fields of each GTF were examined (1000× oil-immersion objective) and each QBC tube was inspected for up to 5 minutes. If no parasites were found, the sample was recorded as negative. The time required for each positive diagnosis was measured carefully with a stopwatch and recorded. In positive QBC specimens, the number, developmental stage, and location of the parasites in the tubes were also recorded. All QBC tubes were inspected within 72 hours of centrifugation.

Two investigators were individually responsible for microscopy: one for GTFs and the other for QBC tubes. Specimens were examined separately and results recorded without knowledge of the outcome of the other test. All specimens with discordant results were re-examined by a senior microscopist, and a final random check was made by one of us (W.X.-Z.).

Results
The results obtained with the GTF technique were taken as the standard of comparison (i.e. GTF was considered to represent a “true” diagnostic test for vivax malaria). The prevalence of malaria in the study area and the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the QBC technique were calculated as follows:

Prevalence = \( \frac{\text{No. of positives by GTF}}{\text{Total no. of samples examined}} \)

\[
\text{Sensitivity} = \frac{\text{No. of positives by both GTF and QBC}}{\text{No. of positives by GTF}}
\]

\[
\text{Specificity} = \frac{\text{No. of negatives by both GTF and QBC}}{\text{No. of negatives by GTF}}
\]

\[
\text{PPV} = \frac{\text{Sensitivity} \times \text{prevalence}}{(\text{Sensitivity} \times \text{prevalence}) + (1 - \text{specificity}) \times (1 - \text{prevalence})}
\]

\[
\text{NPV} = \frac{\text{Specificity} \times (1 - \text{prevalence})}{\text{Specificity} \times (1 - \text{prevalence}) + (1 - \text{specificity}) \times \text{prevalence}}
\]

The concordance of the tests was calculated as follows:

\[
\text{Concordance} = \frac{\text{No. of positives and negatives by both GTF and QBC}}{\text{Total no. of samples examined}}
\]
The values obtained were multiplied by 100 and are reported as percentages.

With reference to the data shown in Table 1, the following values were calculated:

- Prevalence of malaria = 23.6%;
- Sensitivity of the QBC technique = 87.2%;
- Specificity of the QBC technique = 95.0%;
- PPV of the QBC technique = 84.3%;
- NPV of the QBC technique = 96.0%; and
- Concordance of the tests = 93.1%.

These test parameters were also calculated on the basis of the samples collected in hospitals from suspected malaria patients. With reference to the data shown in Table 2, the following values were calculated:

- Prevalence of malaria = 87.2%;
- Sensitivity of the QBC technique = 99.0%;
- Specificity of the QBC technique = 92.9%;
- PPV of the QBC technique = 98.9%;
- NPV of the QBC technique = 92.8%; and
- Concordance of the tests = 98.2%.

As shown in Table 3, different developmental stages of *Plasmodium vivax* parasites were predominantly found after centrifugation in different layers of the QBC tubes, depending on the specific gravity of the parasitized erythrocytes. In this study, ring forms were found in the lower part of the platelet zone at a proportion significantly higher (*P* < 0.01) than that at which they were found in the upper part of the red-blood-cell layer.

### Field evaluation of QBC technique for malaria diagnosis

<table>
<thead>
<tr>
<th>Developmental form:</th>
<th>Ring form</th>
<th>Trophozoite</th>
<th>Schizont</th>
<th>Gametocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of times observed in positive tubes</td>
<td>27</td>
<td>10</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Proportion in different layers:</td>
<td>33.3</td>
<td>20.0</td>
<td>0.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Red blood cell (%)</td>
<td>3.7</td>
<td>20.0</td>
<td>0.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Granulocyte (%)</td>
<td>3.7</td>
<td>50.0</td>
<td>100.0</td>
<td>58.3</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>59.3</td>
<td>10.0</td>
<td>0.0</td>
<td>25.0</td>
</tr>
</tbody>
</table>

* Quantitative buffy coat.
(i.e. GTF (median, 10 min; range, 1 sec–30.0 min)) in diagnosis of vivax malaria.

Discussion

In conjunction with the national malaria control programme in China, more than 18.5 million patients must be examined for malaria twice a year, and one-tenth of these are in Sichuan Province. Although microscopic examination of GTFs is reliable and is currently the cornerstone method (7) for diagnosis of malaria, it requires a great deal of time and training for both the preparation and examination of slides. Moreover, it can become a tedious routine procedure which is burdensome for antimalaria technicians and primary health workers, especially in areas of low parasitaemia and in the surveillance of vivax malaria in controlled areas where the danger of a sudden outbreak nonetheless exists. Therefore, it is useful to consider new tools and to evaluate and select better methods for the rapid diagnosis of vivax malaria for use as alternatives to conventional GTF microscopy.

In this study, all positive diagnoses were confirmed to be of vivax malaria. The results indicate that although the QBC technique is quite specific, it is less sensitive for detecting vivax than falciparum malaria under field conditions (2–5). One explanation may be that the specific gravity of the erythrocyte parasitized by the late developmental stage of P. vivax is approximately equal to or less than 1.074 g/ml (6), whereas that parasitized by P. falciparum is approximately equal to or greater than 1.088 g/ml (8). Therefore, it is understandable that some P. vivax parasites may overlap with leukocytes after centrifugation and may be obscured by concentrated fluorescent granulocytes. However, the sensitivity of the QBC technique was found to correlate with the grade of parasitaemia in this study. In local hospitals, where most patients were suspected to have active malaria, we found that the sensitivity of QBC technique for positive diagnosis was significantly increased.

The distribution of P. vivax parasites in centrifuged QBC tubes was slightly different from that usually observed for P. falciparum parasites (2). Of interest is that frequently the P. vivax ring forms, and sometimes the gametocytes, were found in the platelet zone close to the interphase between the platelet and monocyte zones, where the parasites display typical fluorescence patterns and can be easily detected. Furthermore, as the QBC sample tubes were returned to the university laboratory and stored upright at 4°C, we re-examined positive tubes many times for teaching and training purposes, and found that most specimens, except contaminated ones, were still good weeks later.

The rapidity with which reliable diagnosis can be made, even under field conditions, is one of the major advantages of the QBC technique. In addition, it requires less training and experience than conventional GTF microscopy. Its chief drawback is its high cost, which may limit its use in developing countries. Although the manufacturer now offers a new generation of QBC equipment at lower prices (as used in the present study) it is still too expensive for county-level hospitals in China to obtain.

More recently, however, some investigators have developed new tools for the rapid diagnosis of malaria with more economical equipment and simpler procedures (9, 10).

Acknowledgements

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Résumé

Technique QBC de diagnostic rapide du paludisme à vivax: évaluation sur le terrain

La technique de base pour le diagnostic exact de l'infection paludéenne est l'examen d'une goutte épaisse colorée au Giemsa (GTF). Cette technique longue et fastidieuse pose toutefois des problèmes, notamment dans les régions de faible parasitémie. Certains chercheurs ont récemment démontré qu'il était possible de diagnostiquer le paludisme par examen direct d'échantillons de sang après centrifugation. Cette technique (quantitativeuffy coat ou QBC) repose sur le dénombrement des parasites dans la couenne du sang.

La technique QBC a été comparée au GTF sur le terrain dans les districts de Junlian et de Mingshan (Sichuan, Chine) pour le diagnostic rapide du paludisme à vivax. Des prélèvements de
sang ont été réalisés chez 364 volontaires recrutés parmi les habitants et chaque échantillon a été examiné par les deux techniques. Pour la technique GTF (10µl de sang), l'examen au microscope à immersion dans l'huile a porté sur un maximum de 300 champs et pour le QBC, la durée d'inspection de chaque tube a été limitée à 5 minutes.

Le GTF a donné 86 échantillons positifs et 278 négatifs, et le QBC, 89 échantillons positifs et 275 négatifs. En prenant les résultats du GTF comme valeurs de référence, on obtient pour le QBC une sensibilité de 87,2% et une spécificité de 95,0%; la concordance entre les deux techniques est de 93,1%. La durée médiane pour obtenir un diagnostic positif avec le QBC (1,12 minutes) est de 11% de celle nécessaire avec le GTF, ce qui est important du point de vue de la rapidité du diagnostic.

La distribution des différents stades de développement de Plasmodium vivax a également été examinée dans les tubes de sang centrifugé: toutes les formes à l'exception des schizontes ont pu être retrouvées à la base de la zone des plaquettes (à l'interface entre la couche de monocytès et la couche de plaquettes), en particulier les formes annulaires.

La technique QBC offre donc une possibilité de diagnostic rapide sur le terrain avec une bonne sensibilité et une bonne spécificité. De ce point de vue, elle est particulièrement adaptée aux activités de surveillance et de contrôle des épidémies. Des considérations de coût peuvent toutefois limiter son applicabilité dans les pays en développement, tout au moins au coût actuel.

References