SHORT REPORT: DETECTION OF BORRELLA (RELAPSING FEVER) IN RURAL ETHIOPIA BY MEANS OF THE QUANTITATIVE BUFFY COAT TECHNIQUE

FREDERICK C. COBEY, SETH H. GOLDBARG, ROBERT A. LEVINE, AND CURTIS L. PATTON
Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut

Abstract: The diagnosis of louse-borne relapsing fever is commonly made on the basis of the detection of Borrelia spirochetes on Giemsa-stained thin blood films. In the present study, we used acridine orange–coated quantitative buffy coat (QBC) tubes, centrifugation, and fluorescence microscopy to detect Borrelia. Between July and August 1998, we used the QBC technique to diagnose 7 patients with borreliosis who visited a rural clinic in southwest Ethiopia. In laboratory studies that used Borrelia burgdorferi as a model, we detected spirochetes at concentrations as low as 10 organisms/mm$^3$, whereas the number of positive readings assessed by means of stained blood films fell significantly at dilutions below 3,263 organisms/mm$^3$. The greater sensitivity of the QBC technique is important in areas where Borrelia is endemic, as in the Horn of Africa. It may also prove useful in evaluating relapsing fevers in travelers.

Relapsing fever may be found in many areas of the world, including Africa, Asia, and North America. Louse-borne borreliosis due to B. recurrentis is largely restricted to the northeastern parts of Africa, especially Ethiopia, where an estimated 10,000 cases occur annually. The diagnosis of louse-borne relapsing fever in Ethiopia is commonly made by a Giemsa-stained thin blood smear.

During July and August of 1998, we diagnosed 7 out of 200 patients presenting to the Wolkite Health Clinic complaining of fever with Borrelia infections by means of the quantitative buffy coat (QBC) fluorescent technique. After centrifugation of whole blood for 5 min at 12,000 × g according to the QBC protocol, spirochetes were easily visualized with ultraviolet light in the red blood cell layer. These diagnoses were confirmed with Giemsa-stained blood smears. Spirochetes remained morphologically intact, although not viable.

We performed a dilutional analysis by using Borrelia burgdorferi as a model for relapsing fever. Borrelia burgdorferi and B. recurrentis are morphologically similar, both 20 μm long and 0.2 to 0.3 μm wide and, when stained with acridine orange, they fluoresce similarly under ultraviolet light. We counted 32,625 organisms/mm$^3$ in the culture of B. burgdorferi by means of a hemocytometer and acridine orange dye. We serially diluted the organisms with normal saline to obtain the following dilutions: 1/1, 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, and 1/640. Then 0.1-mL aliquots from each of these saline dilutions were added to 0.9 mL of whole blood, obtaining the final dilutions: 1/10, 1/100, 1/200, 1/400, 1/800, 1/1,600, 1/3,200, and 1/6,400. A whole-blood control was made without organisms. While blinded to the individual concentrations, we examined each of the 8 dilutions 5 times for a maximum of 10 min by use of both Wright-stained blood smears and acridine orange–stained QBC microhematocrit tubes. We used the McNemar chi-square test to analyze the grouped data from the dilutions to document overall statistical significance of the study. Statistical significance for individual dilutions could not be calculated because of the small number (n = 5) per set.

Borrelia can be seen fluorescing in the red blood cell layer in the QBC microhematocrit tube. During the serial dilution, we observed that at lower concentrations, most B. burgdorferi were concentrated near the bottom of the red blood cell layer, in contrast to malarial parasites, which are commonly found near the buffy coat. The QBC in the dilutional series of B. burgdorferi was able to detect spirochetes in human blood down to a concentration of 10 organisms/mm$^3$, in comparison to the Wright-stained blood film, which did not detect organisms < 82 organisms/mm$^3$. The proportion of positive readings found by means of blood film fell significantly after dilutions below 3,263 organisms/mm$^3$, in contrast to the QBC, the accuracy of which fell only at dilutions of < 82 organisms/mm$^3$ ($P < 0.0005$) (Tables 1 and 2).

Louse- and tick-borne relapsing fever are contracted in many areas of the world, including Africa, Asia, and North America. During epidemics, the untreated case fatality rate may reach up to 70%. Travelers returning with relapsing fever from areas where relapsing fever is endemic are often misdiagnosed. Relapsing fever frequently presents clinically like malaria, and because spirochetes may be difficult to find by use of light microscopy, the diagnosis of borreliosis may be missed.

Fluorescence was first used to detect Borrelia in 1983 with acridine orange–stained blood smears. Several studies have shown that the use of the QBC is a more sensitive technique than Giemsa-stained blood smears for detecting malaria. Two published studies have shown that the QBC can be used in the detection of tick-borne relapsing fever. One study also identified a case of relapsing fever in Ethiopia by use of the QBC technique while the researchers were looking for malaria.

Our experience with the QBC in the field and our serial dilution of Borrelia bergdorferi as a model for Borrelia causing relapsing fever both suggest that the QBC technique represents a more rapid and sensitive method for detecting spirochetes than traditional stained peripheral blood smears.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>QBC positive</th>
<th>QBC negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright-stained blood smear, positive</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Wright-stained blood smear, negative</td>
<td>23</td>
<td>10</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>

* Pooled data from dilutional series comparing the sensitivity of quantitative buffy coat (QBC) to Wright-stained blood smears in the detection of Borrelia burgdorferi ($P < 0.0005$).
Table 2

Data regarding detection of Borrelia by dilutional series*

<table>
<thead>
<tr>
<th>Organism/mm³</th>
<th>QBC 5/5</th>
<th>5/5</th>
<th>5/5</th>
<th>82</th>
<th>41</th>
<th>20</th>
<th>10</th>
<th>5</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>QBC</td>
<td>3.263</td>
<td>326</td>
<td>163</td>
<td>82</td>
<td>41</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Wright-stained blood smear</td>
<td>5/5</td>
<td>1/5</td>
<td>0/5</td>
<td>1/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* The denominator is the number of times the given dilution was examined with either quantitative buffy coat (QBC) or blood smear. The numerator is the number of times the technique revealed a positive result.

The sensitivity of the QBC is important in areas where Borrelia is endemic, as in the Horn of Africa, where louse-borne relapsing fever is a significant cause of morbidity and mortality. It may also prove useful in evaluating relapsing fevers in travelers returning from regions in which Borrelia is endemic.

Acknowledgments: We thank the Becton Dickinson Company for their generous contribution of a QBC system.

Financial support: This research was supported by the Wilbur Downs Fellowship and the Committee on International Health at the Yale University School of Medicine.

Authors’ addresses: Frederick C. Cobey, Seth H. Goldbarg, and Curtis L. Patton, Department of Epidemiology and Public Health, and Robert A. Levine, Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT.

Reprint requests: Frederick C. Cobey, 4440 Garfield St. N.W., Washington, DC 20007 (e-mail: cobeyfc@BIOMED.MED.YALE.EDU).

REFERENCES


