SHORT REPORT: DIAGNOSIS OF TICK-BORNE RELAPSING FEVER BY THE QUANTITATIVE BUFFY COAT FLUORESCENCE METHOD

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Abstract. The quantitative buffy coat (QBC) parasite detection method is a sensitive and specific tool for the diagnosis of malaria parasites. It is also useful for the diagnoses of other hemoparasites, including Trypanosoma, Babesia, and Leptospira. We report a case of relapsing fever diagnosed by this technique in a short-term traveler from Senegal. The diagnosis was confirmed by the standard Giemsa hemoscopy and by the identification of significant titers of antibodies to Borrelia spp. of tick-borne relapsing fevers by specific immunofluorescence and Western blot tests. The QBC technique seems to be useful in the diagnosis of tick-borne relapsing fever in blood samples and should be included in the management of fever in the traveler returning from tropical regions.

The quantitative buffy coat (QBC) parasite detection method is based on the centrifugal layering of parasites into a 60-μl blood capillary tube containing acridine orange dye. The test system was initially designed to simplify the diagnosis of malaria. For this disease, it has proved to be as sensitive and specific as the classic thick blood film hemoscopy. The advantage of the QBC technique is in the shorter time required to observe specimens. It is useful for the diagnoses of other hemoparasites, including Trypanosoma, Babesia, and Leptospira. To our knowledge, this is the first case of relapsing fever diagnosed by the QBC fluorescence method.

A 51-year-old Senegalese man developed intermittent fever and body weakness four days after returning from a three-month stay in Dakar, Senegal. He was later admitted to the University Teaching Hospital in Brescia, Italy for a fever of 10 days duration. The results of a physical examination was unremarkable, and the only symptoms he complained of were headache and asthenia. Among the baseline examinations, a blood film was prepared and 60 μl of peripheral blood was taken for detection of hemoparasites. Giemsa-stained thick and thin blood films were negative for malaria parasites. A large number (approximately 10 per field at 1,000×) of actively motile spirochetes were visualized by fluorescence microscopy on a fresh peripheral blood sample processed by the QBC technique. The organisms (Figure 1A) appeared as fluorescent, greenish-yellow, filamentous bodies with 8–10 regularly spaced spirals that clearly stained against the dark background. The parasites were concentrated just above the platelet stratum of the buffy coat, to the University Teaching Hospital in Brescia, Italy for a fever of 10 days duration. The results of a physical examination was unremarkable, and the only symptoms he complained of were headache and asthenia. Among the baseline examinations, a blood film was prepared and 60 μl of peripheral blood was taken for detection of hemoparasites. Giemsa-stained thick and thin blood films were negative for malaria parasites. A large number (approximately 10 per field at 1,000×) of actively motile spirochetes were visualized by fluorescence microscopy on a fresh peripheral blood sample processed by the QBC technique. The organisms (Figure 1A) appeared as fluorescent, greenish-yellow, filamentous bodies with 8–10 regularly spaced spirals that clearly stained against the dark background. The parasites were concentrated just above the platelet stratum of the buffy coat.

Figure 1. Spirochaetes seen in peripheral blood stained by the quantitative buffy coat technique (A, fluorescence microscopy, magnification × 60) and with Giemsa (B, optical microscopy, magnification × 100).
beneath the plasma layer. Based on the epidemiologic data, clinical picture, and the result of the QBC test, a diagnosis of tick-borne relapsing fever was made and the patient was treated with tetracycline for seven days. Fever disappeared on the third day, and the patient was discharged. Six months after treatment, he is symptomless and in good health. Serologic test results for leptospirosis, syphilis, and a commercially available immunofluorescence assay (IFA) for *Borrelia* were negative. Significant titers of antibodies to *B. parkeri*, *B. hermsii*, and *B. coriaceae* were detected by specific IFA and Western Blot tests. Gram staining of a peripheral blood sample obtained during the acute phase was later re-evaluated: one spirochaetal parasite was identified (Figure 1B) after 45 min of observation.

The role of the QBC technique in the diagnosis of human malaria parasites is well established. For this reason, QBC hemoscopy is routinely performed at our referral center as part of a diagnostic procedure for patients with fever and a history of a recent stay in tropical areas. This procedure is potentially effective in the identification of *Plasmodia*, as well as other hemoparasites, including *Borrelia*. Based on the epidemiologic data, *Borrelia crocidurae* is the most likely causative agent of relapsing fever in our patient. The serologic response demonstrated to other *Borrelia* species was interpreted as an interspecific cross-reactivity. The diagnoses of tick-borne relapsing fever rely on direct examination of Giemsa-stained peripheral blood films. However, the sensitivity of this technique is low. In the case we describe, the spirochetes reached a much higher concentration in the QBC tube and the organisms were easily recognized by their active motility. Different from malaria parasites, which are seen between the granulocyte and the red blood cell layer, spirochetes concentrate above the platelet layer of the expanded buffy coat. A similar situation has been reported for African trypanosomes. Diagnosis of tick-borne relapsing fever is important because untreated patients experience relapses of illness and severe complications, such as meningoencephalitis, can ensue. The QBC technique seems to be useful in the diagnosis of this condition and should be included in the management of fever in the traveler returning from tropical regions.

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