Successful in vitro cultivation of *Borrelia duttonii* and its comparison with *Borrelia recurrentis*.


**ABSTRACT** *Borrelia duttonii*, the cause of East African tick-borne relapsing fever, has until now been refractory to growth in laboratory media. This spirochaete has only be propagated in mice or by tissue culture, restricting both yield and purity of cells available for research. The successful isolation of five clinical isolates of *B. duttonii* from patients in Central Tanzania and their comparison with *Borrelia recurrentis* is reported. Electron microscopy revealed spirochaetal cells with pointed ends, a mean wavelength of 1.8 microns with an amplitude of 0.8 micron, similar to the findings for *B. recurrentis*. Cells contained 10 periplasmic flagella inserted at each end of the spirochaete, again comparable with the counts of 8-10 flagella found in *B. recurrentis*. PFGE revealed a chromosome of approximately 1 Mb, a large plasmid of approximately 200 kb, and a small plasmid of 11 kb in all strains of *B. duttonii* and in *B. recurrentis*. *B. duttonii* possessed a further 7–9 plasmids with sizes ranging from 20 to 90 kb. In two isolates of *B. duttonii*, the profiles were identical. In contrast, all 18 isolates of *B. recurrentis* fell into one of five plasmid patterns with 3–4 plasmids ranging from 25 to 61.5 kb in addition to those of 11 and 200 kb described above. Analysis of the SDS-PAGE profiles of *B. duttonii* strains revealed a high-molecular-mass band of
33.4–34.2 kDa in four strains (variable large protein, VLP) and a low-molecular-mass band of 22.3 kDa in the remaining strain (variable small protein, VSP). This resembles the protein profiles found in *B. recurrentis*. The G+C ratio of *B. duttonii* was 27.6 mol%. Nucleotide sequence of the *rrs* gene (16S rRNA) from four *B. duttonii* isolates revealed 100% identity among these strains and 99.7% homology with three strains deposited by others in GenBank. The *rrs* gene of eight representative clinical isolates of *B. recurrentis* confirmed their close similarity with *B. duttonii*.

抄録 ダニ媒介性回帰熱の起因菌である*Borrelia duttonii*は、今まで培地での培養が不可能であったため研究が進展しなかった。我々はタンザニアの回帰熱患者から*B. duttonii* 5株の培養に成功し、これら分離株の性状について検討した。電子顕微鏡像、P FGE、DNA相同性、SDS-PAGE、G+C含量、16S rRNA 遺伝子の塩基配列の解析を行い、シラミ媒介性ペリリア*B. recurrentis*との比較を行った結果、*B. duttonii*は*B. recurrentis*と非常に類似していることが確かめられた。

* Department of Medical Microbiology, Charing Cross Hospital, London, United Kingdom

** Department of Histopathology, Charing Cross Hospital, London, United Kingdom

*** The Wellcome Trust Centre for the Epidemiology of Infectious Disease, University of Oxford, United kingdom

**** Mvumi Hospital, Dodoma, Tanzania