An epidemiological study of tick-borne relapsing fever in Dodoma District, Central Tanzania

W.N. KISINZA[1], P.J. McCALL[2], H. MITANI[3], A. TALBERT[4] & M. FUKUNAGA[3]
[1]*National Institute for Medical Research, P. O. Box 9653 Dar es Salaam, Tanzania*
[2]*Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK*
[3]*Mvumi Hospital, P.O. Box 32, Dodoma, Tanzania*
[4] *Fukuyama University, Fukuyama, Hiroshima 729-0292, Japan*

Abstract: The Muungano village population in central Tanzania was investigated for Borrelia spirochetes by blood slide examination and polymerase chain reaction (PCR). PCR was highly sensitive in detecting infections when compared to microscopy. Using PCR, Borrelia species were identified in 11.1% of children with fever and in 4.2% apparently healthy children. Genotyping the spirochetes from 18 infected children identified *Borrelia duttonii* and a new unnamed species, not known from humans or other vertebrates before. This new species is also incriminated as the cause of tick-borne relapsing fever in the central region of Tanzania.

Introduction

Tick-borne relapsing fever (TBRF) is an acute infectious disease of man caused by spirochetes of the genus *Borrelia,* which is transmitted by soft ticks of the genus *Ornithodoros*. TBRF is characterised by high fever that relapses, drenching sweats, chills, headache, myalgia, arthralgia, abdominal pain, malaise and rash.

TBRF is widely distributed in East and Central Africa. Tick-borne relapsing fever was recently reported to occur in 20% of the districts of Tanzania, being common in north-western and south-western parts of the country (Magesa *et al.,* 2001). The disease is common in central Tanzania, where it can be a significant cause of serious illness, principally in children and pregnant women (Barclay & Coulter, 1990). In Dodoma rural district, TBRF is ranked as the 3rd most important vector-borne disease (Magesa *et al.,* 2002). In endemic villages in the district, house infestations with the *Ornithodoros sp.,* tick can be very high up to 88% (Talbert *et al.,* 1998) and *Borrelia sp.* infection rates in these ticks may be over 60% (Fukunaga *et al.,* 2001). Previous screening of ticks from infested houses for the presence of *Borrelia duttonii*, using the Polymerase Chain Reaction (PCR), revealed a new species of *Borrelia* (Fukunaga *et al.,* 2001). The objective of this study was to determine the prevalence of Borrelia infection among the population of Muungano village in Dodoma, central Tanzania.

Materials and methods

This study was done at Muungano village in Dodoma District of central Tanzania. The village is located approximately 12km north-east of Mvumi Hospital, 40km south-east of Dodoma Municipality. TBRF is known to be endemic in this area, where it is one of the main causes of paediatric illnesses.

Blood samples were taken from three groups randomly selected from the population in Muungano during household surveys in October and November 2002. Finger prick blood samples were taken from children with fever (axillary temperature >37□C) associated with vomiting, chilling, joint/ back pain or headache), pregnant women, and a random sample of afebrile, asymptomatic children. Thick blood smears were prepared and dried at the village prior to subsequent fixing, Giemsa staining and microscopic examination was done at Mvumi Hospital. People with *Borrelia sp.* infections were treated with procaine penicillin fortified at a dosage of 0.4ml (0.96 mu) by intravenous injection daily for 5 days and followed up (for seven days further). Individuals with *Plasmodium* were informed and directed to the nearest dispensary (Mvumi Makulu) for treatment. Blood was also spotted and dried onto small pieces of filter paper (approximately 5mm diameter; Whatman 3MM), placed within labelled Ependorf tubes with pierced lids and stored within sealed plastic bags containing silica gel. A flagellin gene-based polymerase chain reaction (PCR) was used to detect *Borrelia* species; this highly conserved gene has been shown to be suitable for detection and classification of the genus (Fukunuga *et al.,* 2001). Positive samples were

directly sequenced in both directions using an ABI3100 automated sequencer (Perkin Elmer).

Results

Of the 54 under five-year-old children with fever, 5.6% and 11.1% were found to be infected with *Borrelia* by blood slide and PCR respectively (Table 1). One individual with fever had *Borrelia* detected by microscopy but not by PCR. Although the prevalence of malaria (*Plasmodium falciparum* only) was high, only one individual was found with both *Borrelia* (by PCR) and *Plasmodium* species. Of the apparently healthy children 2.3% and 4.0% were positive for *Borrelia* sp. by microscopy and PCR test, respectively. Thus combined blood slide and PCR screening detected *Borrelia* sp. in 4.9% (15/307) of apparently healthy children. No pregnant women were found to be infected.

Genotyping was successful in 17/19 infections and a similarity matrix and neighbour-joining phylogenetic tree were constructed using the DNASTAR and CLUSTAL W software packages, using published sequence data (Fukunuga *et al.*, 2001). Of the four *Borrelia* types found here, two were identical to *B. duttonii* strains Ly and type B described previously (Fukunaga *et al.*, 2001). The others, types 3 and 5, were identical to *Borrelia* Type C isolated previously from *Ornithodoros sp.* ticks in this area. This is a new unnamed *Borrelia* species, differing from *B. duttonii* and the other Afro-tropical species, *B. recurrentis* and *B. crocidurae*, and phylogenetically closer to the New World *B. hermsii*. Flagellin gene sequences (accession numbers AB105117-AB105133) have been deposited into the DDBJ/ GenBank/ EMBL databases. Of the 17 infections genotyped, 11 were *B. duttonii* and 6 were the unidentified species. A single child presenting with fever was infected with the new unnamed *Borrelia* only. In no cases were both *Borrelia* species found in the same host. All infections responded to treatment without complications.

Table 1: Prevalence of *Plasmodium falciparum* and *Borrelia* species in children with or without fever in Muungano village

	No. of children	Malaria parasite Microscopy (N)	Borrelia species Microscopy (N)	PCR (N)
With fever	54	27.8% (15)	5.6% (3)	11.1% (6)
Without fever	307	2% (6)	2.3% (7)	4.2% (13)
Total	361	5.8% (21)	12.8% (10)	5.3% (19)

Key: N = number observed

Table 2. Species composition of *Borrelia* in children with or without fever in Muungano Village

Species	With fever	Without fever	Total
Borrelia duttoni type Ly	4	6	10
Borrelia duttoni type 2 (B)	1	0	1
Unknown type 3	1	4	5
Unknown type 5	0	1	1

Discussion

The discovery of this new *Borrelia* species in humans and in ticks collected within houses, in an area where TBRF is endemic indicates that the organisms causing TBRF in Tanzania are more complex than previously believed. Records of TBRF in most villages within this study area demonstrate how serious this infection is for the local population. The disease has always been attributed to *B. duttonii* and the relative importance and the clinical spectrum of the other species needs to be established. Many further questions arise, including the possible existence of animal reservoirs, possible interaction and differences in aetiology and epidemiology and distribution in both species.

This is the first study to use PCR to screen humans for *Borrelia* sp. infections in Africa and demonstrates the significant increase in sensitivity that can be achieved. As detecting TBRF by microscopy is unreliable (Dennis, 1998) and misdiagnosis likely, TBRF may be more common than records indicate. Infection is likely to be even higher than detected here since during the hot seasons when the study was carried out, some individuals were sleeping outdoors, avoiding attack by the indoor-dwelling ticks. At Mvumi Hospital, monthly *Borrelia* sp. slide positivity rates can be over 7% (Mvumi Hospital, unpublished records), which our PCR data indicate as an underestimate.

With *B. crocidurae* in West Africa (Trape *et al.*, 1991), we must now realise the presence of at least three *Borrelia* species. causing TBRF in Africa, making the development of TBRF vaccines a difficult prospect. However, in East Africa at least, the fact that endophilic ticks transmit both species of pathogen indicates that vector control for TBRF prevention (Talbert *et al.*, 1998) is still a viable method.

Acknowledgements

We thank the people of Muungano for their permission to carry out the study and their cooperation. We are grateful to Mvumi Hospital superintendent for facilitating this work. We thank Ms Asha Abdi and Mr Stafford Sanya for their excellent field assistance. The ethical committees of the Tanzanian National Institute for Medical Research and Liverpool School of Tropical Medicine granted ethical clearance. The study was funded by an award from the Sir Halley Stewart Trust, Cambridge, UK.

References

Barclay, A.J.G. & Coulter, J.B.S. (1990) Tick-borne relapsing fever in central Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **84**, 852-856.

Dennis, D.T. (1998) Borreliosis (relapsing fever). In S.R. Palmer, E.J.L. Soulsby & D.T. Simpson (eds) *Zoonoses: Biology, Clinical Practice and Public Health Control*. Oxford University Press, pp. 17-21.

Fukunaga, M., Ushijima, Y., Aoki, Y. & Talbert, A. (2001) Detection of *Borrelia duttonii*, a tick-borne relapsing fever agent in central Tanzania, within ticks by flagellin gene-based nested polymerase chain reaction. *Vector Borne Zoonotic Diseases*, **1**, 331-338.

Magesa, S.M., Mboera, L.E.G., Mwisongo, A.J., Kisoka, W.J., Mubyazi, G.M., Malebo, H., Senkoro, K.P., Mcharo, J., Makundi, A.J., Kisinza, W.N., Mwanga, J., Mushi, A.K., Hiza, P., Malecela-Lazaro, M.N. & Kitua, A.Y. (2001) Major health problems in some selected districts of Tanzania. *Tanzania Health Research Bulletin* **3**, (2), 10-14.

Soneshine, D.E. (1993) Ecology of Nidicolous Ticks. In: *Biology of Ticks*. Oxford University Press, pp 66-70.

Talbert, A., Nyange, A. & Molteni, F. (1998) Spraying tick-infested houses with lambda-cyhalothrin reduces the incidence of tick-borne relapsing fever in children under five years old. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **92**, 251-253.

Trape, J.F., Duplantier, J.M., Bouganali, H., Godeluck, B., Legros, F., Cornet, J.P. & Camicas, J.L. (1991) Tick-borne borreliosis in West Africa. *The Lancet*, **337**, 473-475.