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Genetic diversity of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks and small ruminants in The Gambia determined by restriction fragment profile analysis

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Abstract

Understanding genetic diversity of *Ehrlichia ruminantium* in host and vector populations is an important prerequisite to controlling heartwater by vaccination in traditional livestock systems in sub-Saharan Africa. We carried out a study in two phases: (i) evaluating the usefulness of the PCR-RFLP assay based on the *map*1 coding sequence of *E. ruminantium* as a discriminatory tool to characterise genetic diversity, (ii) applying the technique to field samples from *Amblyomma variegatum* ticks and small ruminants to characterise genotypic diversity of the organism in three main agroecological zones of The Gambia, Sudano-Guinean (SG), Western Sudano-Sahelian (WSS) and Eastern Sudano-Sahelian (ESS). Restriction fragment length polymorphisms were observed among different strains of *E. ruminantium* supporting the usefulness of the PCR-RFLP technique for studying genetic diversity of the organism. Restriction enzyme *map1* profile analysis indicated the presence in The Gambia of multiple genotypes (at least 11) of *E. ruminantium* with sites in the WSS and SG zones showing comparatively high number of diverse genotypes. Profiles similar to the Kerr Seringe genotype (DQ333230) showed the highest distribution frequency, being present at sites in all three agroecological zones, thereby making the strain a suitable candidate for further characterisation in cross-protection studies. An additional three genotypes showed relatively high distribution frequency and were present in all three zones making them equally important for isolation and subsequent characterisation. The study demonstrated the occurrence of mixed infections with *E. ruminantium* genotypes in ruminants and ticks.

Keywords: *Ehrlichia ruminantium*; *map1*; Restriction fragment length polymorphism; Genetic diversity; Genotypes; The Gambia

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1. Introduction

Heartwater is a rickettsial disease of domestic and wild ruminants caused by *Ehrlichia* (formerly *Cowdria*) *ruminantium* (Dumler et al., 2001). The organism is transmitted by ticks of the genus *Amblyomma*; the major vector in West Africa is *Amblyomma variegatum*, which is distributed in most parts of sub-Saharan Africa and on some islands in the Caribbean (Walker and Olwage, 1987). Heartwater represents a significant obstacle to improvement of livestock production in the tropics and subtropics with mortality rates ranging from 20% to 90% in susceptible animals (Uilenberg, 1983). Small ruminants are particularly at risk from the disease.

Different genotypes of *E. ruminantium* (Allsopp et al., 1997) were found using PCR and sequencing of the V1 loop (Neefs et al., 1993) small-subunit ribosomal RNA gene and random amplified polymorphic DNA and southern blotting (Perez et al., 1997); and stocks with differing immunogenicity exist in the field (Du Plessis et al., 1989). In The Gambia, frequent cases of mortality due to heartwater has been observed in indigenous small ruminants upon translocation from the eastern part of the country to the western part and potential antigenic diversity between different stocks of *E. ruminantium* in the different locations was considered a possible cause (Faburay et al., 2005). Over the past five decades, efforts to control heartwater through development of vaccines have been considerably hampered by the presence of a wide diversity of *E. ruminantium* stocks in the field, which demonstrated phenotypic differences. It is therefore essential to have information on the variety and distribution of stocks within a target area prior to initiating or planning any large-scale vaccination or disease control programmes. At present there is no simple and reliable method for the molecular typing of different *E. ruminantium* stocks (Jongejan and Bekker, 1999). The *map1* gene of *E. ruminantium* represents an ideal target for genotypic characterisation (Allsopp et al., 1999) as it shows a high degree of sequence polymorphisms between isolates (Allsopp et al., 2001; Reddy et al., 1996), does not vary during host–tick passages (D. Martinez, unpublished) and is conserved in all *E. ruminantium* isolates from different geographic regions examined so far (Allsopp et al., 2001; Barbet et al., 1994; Van Vliet et al., 1994). In the present study, we used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique (Geysen et al., 2003) on the *map1* gene to characterise genetic diversity of *E. ruminantium* in ticks and small ruminants in The Gambia. This study was carried out in two phases: (i) evaluating the usefulness of a PCR-RFLP assay of the *map1* coding sequence of *E. ruminantium* to distinguish between different isolates and (ii) characterisation of genetic diversity of *E. ruminantium* in the tick vector and small ruminant hosts at selected sites in the three principal agroecological zones of The Gambia.

2. Materials and methods

2.1. Study sites

2.1.1. Sudano-Guinean zone (SG)

Sampling sites (13°43′N, 16°72′W) in the Sudano-Guinean zone were located within the 900 and 1210 mm of rainfall isohyets. Maximum daily temperatures range from 26 to 32 °C (Climatological Unit, Dept. of Water Resources, The Gambia). The vegetation is savannah-woodland or woodland in certain areas, with *Acacia* spp., *Cordia* spp. and *Elaeis guineensis* predominating lowland ecologies. In some areas around the coast, the vegetation is characterised by humid tropical forest vegetation.

2.1.2. Western Sudano-Sahelian zone (WSS)

Sampling sites (13°20′N, 16°01′W) in the Western Sudano-Sahelian zone received an average of 800 mm of rainfall isohyets with maximum daily temperatures ranging from 28 to 38 °C. The vegetation is composed of degraded savannah woodland interspersed with natural unimproved grasslands dominated by *Andropogon gayanus* and *Meriscus* spp., and also with trees and farmland. The lowland tree vegetation consists principally of low and high mangroves.

2.1.3. Eastern Sudano-Sahelian zone (ESS)

Annual precipitation at the sampling sites (13°27′W, 14°40′N) in the Eastern Sudano-Sahelian zone averages 700 mm of rainfall isohyets with maximum temperatures ranging from 30 to 40 °C. The vegetation is mainly open savannah interspersed with trees, grasses and arable farmland. Towards the river, riparian woodland,