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Short communication

Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequences

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Abstract

Anaplasma marginale is a tick-borne pathogen of cattle that causes the disease bovine anaplasmosis worldwide. Major surface proteins (MSPs) are involved in host–pathogen and tick–pathogen interactions and have been used as markers for the genetic characterization of *A. marginale* strains and phylogenetic studies. MSP1a is involved in the adhesion and transmission of *A. marginale* by ticks and varies among geographic strains in the number and sequence of amino-terminal tandem repeats. The aim of this study was to characterize the genetic diversity of *A. marginale* strains collected from countries in North and South America, Europe, Asia, Africa and Australia, inclusive of all continents. In this study, we characterized 131 strains of *A.*

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marginale using 79 MSP1a repeat sequences. These results corroborated the genetic heterogeneity of *A. marginale* strains in endemic regions worldwide. The phylogenetic analyses of MSP1a repeat sequences did not result in clusters according to the geographic origin of *A. marginale* strains but provided phylogeographic information. Seventy-eight percent of the MSP1a repeat sequences were present in strains from a single geographic region. Strong (≥80%) support was found for clusters containing sequences from Italian, Spanish, Chinese, Argentinean and South American strains. The phylogenetic analyses of MSP1a repeat sequences suggested tick–pathogen co-evolution and provided evidence of multiple introductions of *A. marginale* strains from various geographic locations worldwide. These results contribute to the understanding of the genetic diversity and evolution of *A. marginale* and tick–pathogen interactions.

Keywords: Anaplasmosis; Major surface protein; Evolution; Tick; Genetics; Phylogenetic analysis

1. Introduction

Anaplasma marginale (Rickettsiales: Anaplasmataceae) is the causative agent of bovine anaplasmosis worldwide (Kocan et al., 2004). Ticks are biological vectors of *A. marginale* but the pathogen is often transmitted mechanically to susceptible cattle by blood-contaminated mouthparts of biting flies or fomites (Kocan et al., 2003). These obligate intracellular organisms replicate in membrane-bound parasitophorous vacuoles in bovine erythrocytes or tick cells. Both cattle and ticks become persistently infected with *A. marginale* and thus serve as reservoirs of infection (Kocan et al., 2003, 2004).

Many geographic strains of *A. marginale* have been identified, which differ in biology, genetic characteristics and transmissibility by ticks (de la Fuente et al., 2001a, 2005). The genetic diversity of *A. marginale* strains has been characterized using major surface protein (MSP) genes that are involved in interactions with vertebrate and invertebrate host cells (de la Fuente et al., 2005). These genes may have evolved more rapidly than other genes because of selective pressures exerted by the host immune system.

MSP1a, encoded by the gene *msp1α*, has thus far been identified only in *A. marginale* despite attempts to clone this gene from other *Anaplasma* spp. (de la Fuente et al., 2005). The *A. marginale* MSP1a has evolved under positive selection pressure and geographic strains of the pathogen differ in molecular weight because of a variable number of tandem 23–31 amino acid repeats (Allred et al., 1990; de la Fuente et al., 2001a, 2003a, 2005). MSP1a was shown to be an adhesin for bovine erythrocytes and tick cells (McGarey and Allred, 1994; McGarey et al., 1994; de la Fuente et al., 2001b), and the adhesion domain was identified on the variable N-terminal region containing the repeated peptides (de la Fuente et al., 2003b). MSP1a was also shown to be involved in the transmission of *A. marginale* by *Dermacentor* spp. ticks (de la Fuente et al., 2001c) and to be differentially regulated in tick cells and bovine erythrocytes (Garcia-Garcia et al., 2004).

Due to the high degree of sequence variation within endemic areas, MSP1a sequence analyses of strains from North and South America, Italy, Israel and Australia failed to provide phylogeographic information (de la Fuente et al., 2005). Nevertheless, the analyses of MSP1a repeat sequences on a global scale may provide phylogenetic and evolutionary information about *A. marginale* strains. Therefore, this study was designed for the global characterization of *A. marginale* using MSP1a repeat sequences of cattle strains from North and South America, Europe, Africa, Asia and Australia. This analysis includes new MSP1a sequences from *A. marginale* strains in Mexico, Argentina, Spain, South Africa and China, as well as MSP1a sequences reported previously from North and South America, Italy, Israel and Australia.

2. Materials and methods

2.1. A. marginale strains

A. marginale strains used in this study and Genbank accession numbers for *msp1α* sequences are listed in Fig. 1. All *A. marginale* strains in this study were originally obtained from naturally infected cattle.