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Serologic and molecular characterization of *Anaplasma* species infection in farm animals and ticks from Sicily☆

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Abstract

Although *Anaplasma marginale* was known to be endemic in Italy, the diversity of *Anaplasma* spp. from this area have not been characterized. In this study, the prevalence of *Anaplasma* spp. antibodies in randomly selected farm animals collected on the island of Sicily was determined by use of a MSP5 cELISA for *Anaplasma* spp. and an immunofluorescence test specific for *Anaplasma phagocytophilum*. Genetic variation among strains of *Anaplasma* spp. from animals and ticks was characterized using the *A. marginale msp1α* and the *Anaplasma* spp. *msp4* genes. Eight species of ticks were collected and tested by PCR. Seropositivity for *Anaplasma* spp. and *A. phagocytophilum* was detected in bovine and ovine samples. All the donkeys were seropositive for *A. phagocytophilum* but not for *Anaplasma* spp. Four *A. marginale* genotypes were identified by *msp4* sequences from bovine and tick samples. Two new genotypes of *Anaplasma ovis* were characterized in sheep. The sequences of *A. phagocytophilum* from three donkeys proved to be identical to the sequence of the MRK equine isolate from California. Six *A. marginale* genotypes were found in cattle and one tick using the *A. marginale msp1α* sequences. All genotypes had four repeated sequences in the N-terminal portion of the MSP1a, except for one that had five repeats. The Italian strains of *A. marginale* contained three repeat sequences that were not reported previously. Definition of the diversity of *Anaplasma* spp. in Sicily reported, herein is fundamental to development of control strategies for *A. marginale*, *A. ovis* and *A. phagocytophilum* in Sicily.

Keywords: Anaplasmosis; *A. marginale*; *A. phagocytophilum*; *A. ovis*

☆ The GenBank accession numbers for *msp4* sequences of *A. marginale*, *A. ovis* and *A. phagocytophilum* strains are AY702917–AY702925 and for *A. marginale msp1α* are AY702926–AY702932.

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1. Introduction

The genus *Anaplasma* (Rickettsiales: Anaplasmataceae) includes three species that infect ruminants: *Anaplasma marginale* (the type species), *Anaplasma centrale* and *Anaplasma ovis* (reviewed by Kocan et al., 2003). Bovine anaplasmosis is caused primarily by *A. marginale* while *A. ovis* is a pathogen of sheep and is not infectious for cattle. *A. centrale*, a less pathogenic organism, is used as a live vaccine for cattle in Israel, South Africa, South America and Australia. As the result of a recent reclassification of the Anaplasmataceae, the genus *Anaplasma* now also includes *Anaplasma phagocytophilum* (formerly *Ehrlichia equi*, *Ehrlichia phagocytophila* and the HGE agent of human granulocytic ehrlichiosis, now recognized as synonymous), which causes a febrile disease in ruminants, and human, equine and canine granulocytic anaplasmosis (Dumler et al., 2001).

Ticks are biological vectors of *Anaplasma* spp., and mammalian, bird or tick hosts with persistent *Anaplasma* spp. infection may serve as natural reservoirs of infection (reviewed by Dumler et al., 2001; Kocan et al., 2003; Rikihisa, 2003).

Many geographic strains of *A. marginale* and *A. phagocytophilum* have been identified which differ in biology, genetic characteristics and/or pathogenicity (Dumler et al., 2001; Massung et al., 2000, 2002, 2003; Stuen et al., 2003; Kocan et al., 2003; Polin et al., 2004; de la Fuente et al., 2005a,b). Genetic diversity has not been widely characterized for *A. ovis*.

Recent research on *Anaplasma* spp. has focused on major surface proteins (MSPs) that are involved in interactions with vertebrate and invertebrate host cells (Kocan et al., 2003; Rikihisa, 2003; Lin et al., 2004; de la Fuente et al., 2005b). Selected MSPs, such as MSP1a, MSP2 and MSP4, have been used to characterize the genetic diversity of *Anaplasma* spp. (reviewed by de la Fuente et al., 2005b). These MSPs, involved in host–pathogen interactions, may have evolved more rapidly than other genes because of selective pressures exerted by the host immune system.

A. marginale is endemic in Sicily and in other regions of the world (reviewed by Kocan et al., 2003) and has been described previously in Italy (Cringoli et al., 2002; Tassi et al., 2002). However, the Italian strains of *Anaplasma* spp. have not been characterized at the molecular level. In this study, we examine the genetic variation among strains of *Anaplasma* spp. obtained from infected cattle, sheep, donkeys and ticks in the island of Sicily in southern Italy using the *A. marginale msp1α* and the *Anaplasma* spp. *msp4* genes.

2. Materials and methods

2.1. Samples

Blood from 50 cattle, 8 sheep and 3 donkeys, plus 88 ticks, were collected in Sicily, Italy for these studies. Blood was collected from randomly selected farm animals mainly in the province of Palermo but also in Trapani, Ancona and Ragusa. Ticks were collected from cattle in the province of Palermo, district of Carleone, and stored in 70% ethanol at room temperature. Sixty-eight adult ticks and 20 nymphs that were pooled from each host were collected. Ticks were identified using morphological keys for Italian Ixodidae (Manilla, 1998). Blood was collected into sterile tubes with and without anticoagulant (lithium heparin) and maintained at 4 °C until arrival at the laboratory. Plasma and serum were then separated after centrifugation and stored at −20 °C.

2.2. Serologic tests for detection of Anaplasma spp

The anaplasmosis cELISA was performed using the *Anaplasma* Antibody Test Kit, cELISA from VMRD Inc. (Pullman, WA, USA) following the manufacturer's instructions. This assay detects serum antibodies against the MSP5 protein of *Anaplasma* spp. (Knowles et al., 1996).

The immunofluorescence test for *A. phagocytophilum* was performed using the IFA Antibody Test Kit from Fuller Laboratories (Fullerton, CA, USA) following the manufacturer's instructions.

2.3. DNA extraction, PCR and sequence analysis

DNA was extracted from blood and tick samples using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, St. Louis, MO, USA). The *A. marginale msp1α* and the *Anaplasma* spp. *msp4* genes