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ScienceDirect
Veterinary Microbiology 119 (2007) 375–381
veterinary microbiology
www.elsevier.com/locate/vetmic

Short communication

Sequence analysis of the *msp4* gene of *Anaplasma ovis* strains

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Received 9 June 2006; received in revised form 17 September 2006; accepted 18 September 2006

Abstract

Anaplasma ovis (Rickettsiales: Anaplasmataceae) is a tick-borne pathogen of sheep, goats and wild ruminants. The genetic diversity of *A. ovis* strains has not been well characterized due to the lack of sequence information. In this study, we evaluated bighorn sheep (*Ovis canadensis*) and mule deer (*Odocoileus hemionus*) from Montana for infection with *A. ovis* by serology and sequence analysis of the *msp4* gene. Antibodies to *Anaplasma* spp. were detected in 37% and 39% of bighorn sheep and mule deer analyzed, respectively. Four new *msp4* genotypes were identified. The *A. ovis msp4* sequences identified herein were analyzed together with sequences reported previously for the characterization of the genetic diversity of *A. ovis* strains in comparison with other *Anaplasma* spp. The results of these studies demonstrated that although *A. ovis msp4* genotypes may vary among geographic regions and between sheep and deer hosts, the variation observed was less than the variation observed between *A. marginale* and *A. phagocytophilum* strains. The results reported herein further confirm that *A. ovis* infection occurs in natural wild ruminant populations in Western United States and that bighorn sheep and mule deer may serve as wildlife reservoirs of *A. ovis*.

Keywords: Anaplasmosis; Major surface protein; Reservoir host; Mule deer; Bighorn sheep; Wildlife

1. Introduction

Anaplasma ovis is an intraerythrocytic rickettsial pathogen of sheep, goats and wild ruminants (Krier and Ristic, 1963; Zaugg, 1987, 1988; Kuttler, 1984; Zaugg et al., 1996; Friedhoff, 1997; Yabsley et al.,

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0378-1135/$ – see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.vetmic.2006.09.011

2005; de la Fuente et al., 2006). This pathogen is classified in the genus *Anaplasma* (Rickettsiales: Anaplasmataceae), along with *A. marginale* (the type species), *A. phagocytophilum* and *A. bovis* which also infect ruminants and *A. platys* that infects dogs (Dumler et al., 2001; Kocan et al., 2004). Ticks of the genus *Dermacentor* are biological vectors of *A. ovis* in the western United States. In the Old World, *A. ovis* is transmitted by *R. bursa* and most likely other ticks (Friedhoff, 1997). Mammalian or tick hosts with persistent infection serve as reservoirs of the pathogen in nature (Kocan et al., 2004).

Anaplasma major surface proteins (MSPs) are involved in interactions with both vertebrate and invertebrate hosts (de la Fuente et al., 2005a; Kocan et al., 2004; Brayton et al., 2006; Dunning Hotopp et al., 2006), and therefore are likely to evolve more rapidly than other genes because they are subjected to selective pressures exerted by host immune systems. The *msp4* gene, the function of which is currently unknown, is part of the MSP2 protein superfamily (de la Fuente et al., 2005a; Brayton et al., 2006). The *msp4* gene and protein sequences have proven useful for phylogenetic studies of *A. marginale* and *A. phagocytophilum* (de la Fuente et al., 2002a, 2005a,b) and for the genetic characterization of *Anaplasma* spp. (reviewed by de la Fuente et al., 2005a).

Many geographic strains of *Anaplasma* have been identified which differ in biology, genetic characteristics and/or pathogenicity (reviewed by de la Fuente et al., 2005a). While the genetic diversity of *A. marginale* and *A. phagocytophilum* has been studied extensively, the genetic diversity for *A. ovis* has not been well characterized due to the lack of sequence information (reviewed by de la Fuente et al., 2005a). Few studies have analyzed *A. ovis msp4* sequences from both domesticated sheep and wild sheep and deer populations with the identification of three different genotypes only (de la Fuente et al., 2002b, 2005c, 2006; Yabsley et al., 2005).

In this study, we evaluated bighorn sheep (*Ovis canadensis*) and mule deer (*Odocoileus hemionus*) from Montana for infection with *A. ovis* by serology and sequence analysis of the *msp4* gene. The four new *msp4* genotypes identified herein were analyzed together with the three sequences reported previously to characterize the genetic diversity of *A. ovis* strains in comparison with other *Anaplasma* spp.

2. Materials and methods

2.1. Animals and sample preparation

During November 2002–October 2005, 83 bighorn sheep were darted and captured in Glacier National Park, MT, USA, using combinations of carfentanil and xylazine, primarily during the October–November and April–May periods. During March 2006, 13 additional bighorn sheep were captured via helicopter net-gunning in adjacent Waterton Lakes National Park, AB, Canada. Samples from 57 of these animals were submitted for serologic testing. Mule deer ($N = 70$) were physically restrained using a net gun fired from a helicopter for the purpose of radio-collaring. These animals were captured between 10 and 14 January 2006 on privately owned land in southeastern Montana. Blood was collected into separate sterile tubes with and without anticoagulant (EDTA) and maintained at 4 °C until processed. Plasma and serum were then separated after centrifugation and stored at −20 °C. DNA was extracted from blood samples of seropositive animals using Tri Reagent (Sigma, St. Louis, MO, USA) and following manufacturer's recommendations. Experiments were performed according to the U.S. laws and approved by the Montana State University ethical committee.

2.2. Anaplasmosis serologic test

Animals were tested for antibodies to *Anaplasma* spp. by the State of Montana Department of Livestock Diagnostic Laboratory (Bozeman, MT, USA) using the cELISA anaplasmosis test (VMRD Inc., Pullman, WA, USA) (Knowles et al., 1996), which detects antibodies to the MSP5 antigen conserved between *A. marginale*, *A. phagocytophilum* and *A. ovis* (Dreher et al., 2005). Percent inhibition values greater than 30% were considered positive as recommended by the manufacturer.

2.3. msp4 PCR and sequence analysis

The *Anaplasma* spp. *msp4* gene was amplified by PCR and sequenced as reported previously (de la Fuente et al., 2002a, 2005b). Briefly, 1 μl (1–10 ng) DNA was used with 10 pmol of each primer (*A. marginale*/*A. ovis*: MSP45: 5′-GGGAGCTCCTATGAATTACAGAGAATTGTTTAC-3′ and MSP43: