# Genetic Characterization of Anaplasma ovis Strains from Bighorn Sheep in Montana

Authors: de la Fuente, José, Atkinson, Mark W., Hogg, John T., Miller, David S., Naranjo, Victoria, et al.

Source: Journal of Wildlife Diseases, 42(2) : 381-385

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-42.2.381

*Journal of Wildlife Diseases*, 42(2), 2006, pp. 381–385


# Genetic Characterization of *Anaplasma ovis* Strains from Bighorn Sheep in Montana

**José de la Fuente,[1,2,6] Mark W. Atkinson,[3] John T. Hogg,[4] David S. Miller,[5] Victoria Naranjo,[2] Consuelo Almazán,[1] Neil Anderson,[3] and Katherine M. Kocan[1]** [1] Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, Oklahoma 74078, USA; [2] Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain; [3] Montana Fish, Wildlife and Parks, 1400 S. 19th St., Bozeman, Montana 59718, USA; [4] Montana Conservation Science Institute, Ltd., 5200 Upper Miller Creek Road, Missoula, Montana 59803, USA; [5] Animal Population Health Institute, Veterinary Teaching Hospital, Colorado State University, 300 West Drake, Fort Collins, Colorado 80523, USA; [6] Corresponding author (email: jose_delafuente@yahoo.com or djose@cvm.okstate.edu)

ABSTRACT: Wildlife reservoir species and genetic diversity of *Anaplasma ovis* (Rickettsiales: Anaplasmataceae) have been poorly characterized. Bighorn sheep (*Ovis canadensis*), captured in Montana from December 2004 to January 2005, were tested for antibodies to *Anaplasma* spp.; the presence of *A. ovis* was determined by the characterization of major surface protein *msp4* sequences. *Anaplasma* antibodies were detected in 25/180 (14%) sampled bighorn sheep and *A. ovis msp4* sequences were amplified by polymerase chain reaction (PCR) and sequenced from 9/23 (39%) of seropositive animals. All animals were negative by PCR for the related pathogens, *Anaplasma phagocytophilum* and *Anaplasma marginale*. All *msp4* sequences identified in the bighorn sheep were identical and corresponded to a single *A. ovis* genotype that was identical to a sheep isolate reported previously from Idaho. The finding of a single genotype of *A. ovis* in this wild herd of bighorn sheep was in contrast to the genetic diversity reported for *A. marginale* in cattle herds in the western United States and worldwide. These results demonstrated that bighorn sheep may be a wildlife reservoir of *A. ovis* in Montana.

*Key words:* *Anaplasma ovis*, anaplasmosis, bighorn sheep, major surface protein, *msp4*, reservoir host.

*Anaplasma ovis* (Rickettsiales: Anaplasmataceae) is an intraerythrocytic rickettsial pathogen of sheep and goats (Friedhoff, 1997). This pathogen is classified in the genus *Anaplasma*, along with *Anaplasma marginale* (the type species) and *Anaplasma phagocytophilum*, which also infect ruminants (Kocan et al., 2003). Ticks of the genus *Dermacentor* are biological vectors of *A. ovis* in the western United States, and persistently infected mammalian or tick hosts may serve as reservoirs of the pathogen (Kocan et al., 2004).

Although *A. ovis* does not infect cattle, this pathogen has been reported to infect wild ruminants (Kuttler, 1984). For example, mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus*), and pronghorn antelope (*Antilocapra americana*) are susceptible to experimental infections with *A. ovis* (Krier and Ristic, 1963; Zaugg, 1987, 1988; Zaugg et al., 1996). Furthermore, mountain goats (*Oreamnos americanus*) and elk captured in Choteau and Madison counties, respectively, in Montana were serologically positive for *Anaplasma* spp. (Atkinson, unpubl. data). Rocky Mountain elk (*Cervus elaphus nelsoni*) can become infected with *A. marginale* (Kuttler, 1984). However, the role of these wild ruminants in the epidemiology of *A. ovis* is unknown.

Bighorn sheep (*Ovis canadensis*) populations seropositive for *Anaplasma* spp. were identified in California, suggesting a role for this species in the epidemiology of *Anaplasma* spp. in the western United States (Goff et al., 1993; Jessup et al., 1993; Crosbie et al., 1997). An *A. ovis* isolate from bighorn sheep was characterized with monoclonal antibodies and DNA probes (Goff et al., 1993), but sequence analysis of pathogen genes was not reported. Bighorn sheep develop clinical disease when experimentally infected with *A. ovis*, suggesting that wild populations may be adversely affected if exposed to the pathogen in nature. Thus, bighorn sheep translocations from areas with *A. ovis*

infection may pose a risk for the spread of this disease (Tibbitts et al., 1992).

Many geographic strains of *Anaplasma* have been identified which differ in biology, genetic characteristics and/or pathogenicity (de la Fuente et al., 2005a). Although the genetic diversity of *A. marginale* and *A. phagocytophilum* (de la Fuente et al., 2005a) has been studied extensively, the genetic diversity of *A. ovis* has not been characterized.

In this study, we evaluated bighorn sheep from Montana for infection with *A. ovis* and characterized the genetic diversity of *A. ovis* strains by sequence analysis of the major surface protein, the *msp4* gene. This gene is involved in host–pathogen interactions and has been used to characterize the genetic diversity of other *Anaplasma* species (de la Fuente et al., 2005a).

Bighorn sheep ($n=180$) were captured by helicopter net gun and physical restraint for radio-collaring and translocation to Utah from December 2004 to January 2005 in Sanders, Lewis and Clark, Teton, and Fergus counties, Montana. Blood was collected into separate sterile tubes with and without anticoagulant (lithium heparin) and maintained at 4 C until processed. Plasma and serum were then separated after centrifugation and stored at −20 C. Animals were tested for antibodies to *Anaplasma* spp. by the State of Montana Department of Livestock Diagnostic Laboratory (Bozeman, Montana, USA) using the cELISA test (VMRD, Inc., Pullman, Washington, 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).

Antibodies to *Anaplasma* spp. were detected in 25 (14%) of bighorn sheep tested. All the animals seropositive for *Anaplasma* were captured in one geographic area, the area around the towns of Paradise and Perma, Sanders County, Montana, from which a total of 36 animals were analyzed from two separate herds. The prevalence of *Anaplasma* antibodies in this area was 69% (25/36).

DNA was extracted from anticoagulated blood samples of 23 seropositive animals using Tri Reagent (Sigma, St. Louis, Missouri, USA) and following manufacturer's recommendations. The *Anaplasma* spp. *msp4* genes were amplified by polymerase chain reaction (PCR) and sequenced as reported previously (de la Fuente et al., 2002a, b). 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: 5′-CCGGATCCTTAGCTGAACAGGAATCTTGC-3′; *A. phagocytophilum*: MAP4AP5: 5′-ATGAATTACAGAGAATTGCTTGTAGG-3′ and MSP4AP3: 5′-TTAATTGAAAGCAAATCTTGCTCCTATG-3′) in a 50-µl volume PCR (1.5 mM $MgSO_4$, 0.2 mM dNTP, 1× AMV/Tfl reaction buffer, 5µl *Tfl* DNA polymerase) employing the Access reverse transcriptase–PCR system (Promega, Madison, Wisconsin, USA). Reactions were performed in an automated DNA thermal cycler (Eppendorf Mastercycler® personal, Westbury, New York, USA) for 35 cycles. After an initial denaturation step of 30 sec at 94 C, each cycle consisted of a denaturing step of 30 sec at 94 C, an annealing for 30 sec at 60 C and an extension step of 1 min at 68 C for *A. marginale/A. ovis* and an annealing-extension step of 1 min at 68 C for *A. phagocytophilum*. Negative control reactions were performed with the same procedures, but adding water instead of DNA to control contamination of the PCR. The program ended by storing the reactions at 4 C. Polymerase chain reaction products were electrophoresed on 1% agarose gels to check the size of amplified fragments by comparison to a DNA molecular weight marker (1 Kb DNA Ladder, Promega). Amplified fragments were resin-purified (Wizard, Promega) and cloned into the pGEM-T vector (Promega) for sequencing both strands by double-stranded dye-termina-

tion cycle sequencing (Core Sequencing Facility, Department of Biochemistry and Molecular Biology, Noble Research Center, Oklahoma State University, Stillwater, Oklahoma, USA). At least two independent clones were sequenced for each PCR. The *msp4* coding region was used for sequence alignment. Multiple sequence alignment was performed using the program AlignX (Vector NTI Suite V 5.5, InforMax, North Bethesda, Maryland, USA) with an engine based on the Clustal W algorithm (Thompson et al., 1994).

The *Anaplasma* spp. *msp4* sequences were amplified and sequenced in nine (39%) of the 23 seropositive samples. The differences in the results of PCR analysis as compared with the serology results may be explained by the sensitivity of the PCR (5 copies *msp4*/ng DNA), which may be lower than the infection level usually found in carrier animals. All sequences corresponded to *A. ovis*. Sequence analysis of *msp4* amplicons demonstrated a single genotype in infected animals, identical to the *A. ovis* Idaho isolate reference sequence (Genbank accession number AF393742; de la Fuente et al., 2002a). *Anaplasma phagocytophilum* and *A. marginale* were not detected in any of the samples by use of the *msp4* PCR.

The absence of *A. marginale* and *A. phagocytophilum* infections in this herd of bighorn sheep may be because of the limited host range of *A. marginale*, which infects primarily cattle and cervids (Kuttler, 1984), the absence of biological and mechanical vectors for these pathogens, and/or the possibility that some *Anaplasma* genotypes may exclude the multiplication of other genotypes as has been described for *A. marginale*, *A. ovis*, and *A. phagocytophilum* strains (de la Fuente et al., 2002b; Stuen et al., 2005).

Genetic diversity of *A. marginale* strains has been described within cattle herds and geographic locations, suggesting that multiple introductions of genetically diverse strains of the pathogen occurred in most geographic areas with further diversification by cattle movement (de la Fuente et al., 2005a). Maintenance of different genotypes by independent transmission events was shown to occur by infection exclusion of *A. marginale* in cattle and ticks, which results in the establishment of a single genotype in most animals (de la Fuente et al., 2005a). Genetic diversity of *A. phagocytophilum* strains also occurs in geographic regions, but ruminant and nonruminant strains of the pathogen can be differentiated by use of the *msp4* sequence (de la Fuente et al., 2005b).

Although the data available for *A. ovis msp4* is limited to a sheep isolate from Idaho (Genbank accession number AF-393742; de la Fuente et al., 2002a), two *A. ovis* genotypes reported from sheep in Sicily, Italy (Genbank accession numbers AY702923 and AY702924; de la Fuente et al., 2005c) and those reported herein, the results from this study demonstrated that, although *A. ovis msp4* genotypes may vary among geographic areas, the variation observed thus far is less than that observed in *A. marginale* and *A. phagocytophilum*. This finding may have resulted from restricted movement of infected hosts. Additionally, the limited host range of *A. ovis* as compared with *A. phagocytophilum* may have also contributed to the lack of genetic diversity of this rickettsia.

The role of bighorn sheep in the epidemiology of *A. ovis* has not been well characterized. The results described herein support the potential for bighorn sheep to serve as wildlife reservoirs of *A. ovis*. The potential influence of bighorn sheep as reservoirs of *A. ovis* on the epidemiology of the disease and animal translocation in the western United States warrants further investigation.

This research was supported by the Endowed Chair for Food Animal Research and the Oklahoma Agricultural Experiment Station Project 1669 (K.M. K.), the Montana Fish, Wildlife and Parks, the Animal Population Health Institute at Colorado State University, and the Foun-

dation for North American Wild Sheep. V. N. is funded by Junta de Comunidades de Castilla, La Mancha, Spain. C. A. is supported by a grant-in-aid from the CONACYT, Mexico and a grant from Pfizer Animal Health, Inc., Kalamazoo, Michigan, USA. We thank Jennifer Southers for technical assistance.

## LITERATURE CITED

CROSBIE, P. R., W. L. GOFF, D. STILLER, D. A. JESSUP, AND W. M. BOYCE. 1997. The distribution of *Dermacentor hunteri* and *Anaplasma* sp. in desert bighorn sheep (*Ovis canadensis*). Journal of Parasitology 83: 31–37.

DE LA FUENTE, J., R. A. VAN DEN BUSSCHE, J. C. GARCIA-GARCIA, S. D. RODRÍGUEZ, M. A. GARCÍA, A. GUGLIELMONE, A. J. MANGOLD, L. M. FRICHE PASSOS, E. F. BLOUIN, AND K. M. KOCAN. 2002a. Phylogeography of New World isolates of *Anaplasma marginale* (Rickettsiaceae: Ehrlichieae) based on major surface protein sequences. Veterinary Microbiology 88: 275–285.

———, J. C. GARCÍA-GARCÍA, E. F. BLOUIN, J. T. SALIKI, AND K. M. KOCAN. 2002b. Infection of tick cells and bovine erythrocytes with one genotype of the intracellular ehrlichia *Anaplasma marginale* excludes infection with other genotypes. Clinical and Diagnostic Laboratory Immunology 9: 658–668.

———, A. LEW, H. LUTZ, M. L. MELI, R. HOFMANN-LEHMANN, V. SHKAP, T. MOLAD, A. J. MANGOLD, C. ALMAZÁN, V. NARANJO, C. GORTÁZAR, A. TORINA, S. CARACAPPA, A. L. GARCÍA-PÉREZ, M. BARRAL, B. OPORTO, L. CECI, G. CARELLI, E. F. BLOUIN, AND K. M. KOCAN. 2005a. Genetic diversity of *Anaplasma* species major surface proteins and implications for anaplasmosis serodiagnosis and vaccine development. Animal Health Research Reviews 6: 75–89.

———, R. B. MASSUNG, S. J. WONG, F. K. CHU, H. LUTZ, M. L. MELI, F. D. VON LOEWENICH, A. GRZESZCZUK, A. TORINA, S. CARACAPPA, A. J. MANGOLD, V. NARANJO, S. STUEN, AND K. M. KOCAN. 2005b. Sequence analysis of the *msp4* gene of *Anaplasma phagocytophilum* strains. Journal of Clinical Microbiology 43: 1309–1317.

———, A. TORINA, S. CARACAPPA, G. TUMINO, R. FURLÁ, C. ALMAZÁN, AND K. M. KOCAN. 2005c. Serologic and molecular characterization of *Anaplasma* species infection in farm animals and ticks from Sicily. Veterinary Parasitology 133: 357–362.

DREHER, U. M., J. DE LA FUENTE, R. HOFMANN-LEHMANN, M. L. MELI, N. PUSTERLA, K. M. KOCAN, Z. WOLDEHIWET, U. BRAUN, G. REGULA, K. D. C. STAERK, AND H. LUTZ. 2005. Serologic cross-reactivity between *Anaplasma marginale* and *Anaplasma phagocytophilum*. Clinical and Diagnostic Laboratory Immunology 12: 1177–1183.

FRIEDHOFF, K. T. 1997. Tick-borne diseases of sheep and goats caused by *Babesia*, *Theileria* or *Anaplasma* spp. Parassitologia 39: 99–109.

GOFF, W., D. STILLER, D. JESSUP, P. MSOLLA, W. BOYCE, AND W. FOREYT. 1993. Characterization of an *Anaplasma ovis* isolate from desert bighorn sheep in southern California. Journal of Wildlife Diseases 29: 540–546.

JESSUP, D. A., W. L. GOFF, D. STILLER, M. N. OLIVER, V. C. BLEICH, AND W. M. BOYCE. 1993. A retrospective serologic survey for *Anaplasma* spp. infection in three bighorn sheep (*Ovis canadensis*) populations in California. Journal of Wildlife Diseases 29: 547–554.

KNOWLES, D., S. TORIONI DE ECHAIDE, G. PALMER, T. MCGUIRE, D. STILLER, AND T. MCELWAIN. 1996. Antibody against an *Anaplasma marginale* MSP5 epitope common to tick and erythrocyte stages identifies persistently infected cattle. Journal of Clinical Microbiology 34: 2225–2230.

KOCAN, K. M., J. DE LA FUENTE, A. A. GUGLIELMONE, AND R. D. MELÉNDEZ. 2003. Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. Clinical Microbiology Reviews 16: 698–712.

———, ———, E. F. BLOUIN, AND J. C. GARCIA-GARCIA. 2004. *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): Recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitology 129: S285–S300.

KRIER, J. P., AND M. RISTIC. 1963. Anaplasmosis. VII. Experimental *Anaplasma ovis* infection in white-tailed deer (*Dama virginiana*). American Journal of Veterinary Research 24: 567–572.

KUTTLER, K. L. 1984. *Anaplasma* infections in wild and domestic ruminants: A review. Journal of Wildlife Diseases 20: 12–20.

STUEN, S., H. DAHL, K. BERGSTROM, AND T. MOUM. 2005. Unidirectional suppression of *Anaplasma phagocytophilum* genotypes in infected lambs. Clinical and Diagnostic Laboratory Immunology 12: 1448–1450.

THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680.

TIBBITTS, T., W. GOFF, W. FOREYT, AND D. STILLER. 1992. Susceptibility of two Rocky Mountain bighorn sheep to experimental infection with *Anaplasma ovis*. Journal of Wildlife Diseases 28: 125–129.

ZAUGG, J. L. 1987. Experimental infections of *Anaplasma ovis* in pronghorn antelope. Journal of Wildlife Diseases 23: 205–210.

———. 1988. Experimental anaplasmosis in mule

deer: Persistence of infection of *Anaplasma marginale* and susceptibility to *A. ovis*. Journal of Wildlife Diseases 24: 120–126.

———, W. L. Goff, W. Foreyt, and D. L. Hunter. 1996. Susceptibility of elk (*Cervus elaphus*) to experimental infection with *Anaplasma marginale* and *A. ovis*. Journal of Wildlife Diseases 32: 62–66.

*Received for publication 6 May 2005.*