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Research article

Isolation of *Bartonella capreoli* from elk

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ABSTRACT

The aim of the present study was to investigate the presence of *Bartonella* infections in elk populations. We report the isolation of four *Bartonella* strains from 55 elk blood samples. Sequencing analysis demonstrated that all four strains belong to *Bartonella capreoli*, a bacterium that was originally described in the wild roe deer of Europe. Our finding first time demonstrated that *B. capreoli* has a wide geographic range, and that elk may be another host for this bacterium. Further investigations are needed to determine the impact of this bacterium on wildlife.

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1. Introduction

The genus *Bartonella* contains at least 20 species and/or subspecies, several of which are important zoonotic agents (Bass et al., 1997; Chomel, 1996a). These Gram-negative bacteria are fastidious, facultatively intracellular, and distributed among a broad range of mammalian hosts. Parasitic arthropods (i.e. blood-sucking), such as fleas, flies, deer keds, mites, and ticks, have been implicated as vectors transmitting bartonellae among different mammalian hosts (Baker, 1946; Garcia-Caceres and Garcia, 1991; Chomel et al., 1996b; Higgins et al., 1996; Welch et al., 1999; Dehio et al., 2004; Halos et al., 2004; Reeves et al., 2006).

Several *Bartonella* species are associated with ruminant hosts. *Bartonella bovis* was originally diagnosed in a cow from France (Bermond et al., 2002), but is now commonly found in cattle from many regions worldwide (Raoult et al., 2005). *B. bovis* is also reported in other ruminant species, including mule deer and elk (Chang et al., 2000). *Bartonella schoenbuchensis* was first isolated from wild roe deer (*Capreolus capreolus*) in Germany (Dehio et al., 2001) and was later reported in French cattle (Rolain et al., 2003) as well as deer keds (*Lipoptena cervi*) collected from roe deer and red deer in Germany (Dehio et al., 2004). *Bartonella capreoli*, a species that is genetically close to *B. schoenbuchensis*, was also first isolated from wild roe deer in France (Bermond et al., 2002) and was found recently in ticks (*Ixodes ricinus*) collected from roe deer in Poland (Bogumiła and Adamska, 2005). Other ruminant-associated *Bartonella* species include *Bartonella chomelii* isolated from French domestic cattle (Maillard et al., 2004) and *Bartonella melophagi* that was isolated from sheep and sheep keds in the U.S. (Bemis and Kania, 2007; Kosoy et al., 2005). Very recently, *Bartonella henselae* was reported to co-infect cattle with *B. bovis* (Cherry et al., 2009).

In the present study, we defined the *Bartonella* species isolated from elk (*Cervus elaphus*) blood and assessed the prevalence of bartonella in elk from Wyoming.

2. Material and methods

2.1. Sample collection

In January–February 2008 and January–February 2009, we collected 55 blood samples from adult female elk. The elk were located on five sites in northwestern Wyoming

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where they are supplementally fed by the Wyoming Game and Fish Department for several months every winter. The primary goal of our sampling was for monitoring brucellosis and new infections that are thought to be driven by pregnant females and males have a limited (or no) role in transmission, and so we only collected adult female samples. We kept samples at −20 °C until they were sent to the Bartonella Laboratory, Centers for Disease Control and Prevention, Fort Collins, CO, for testing.

2.2. Bartonella culturing

Blood was re-suspended to 1:4 in brain heart infusion broth supplemented with 5% amphotericin B that does not affect growth of *Bartonella* bacteria but can reduce the likelihood that bacterial and fungal contaminants would overgrow the slow-growing *Bartonella* bacteria (Kosoy et al., 1997), then plated on heart infusion agar containing 10% rabbit blood and incubated in an aerobic atmosphere with 5% CO_2 at 35 °C for at least 4 weeks. Bacterial colonies were presumptively identified as *Bartonella* based on their morphology. Subcultures of *Bartonella* colonies from the original agar plate were streaked onto secondary agar plates. Pure cultures were harvested and stored in 10% glycerol.

2.3. Verification of Bartonella by PCR amplification

Bartonella isolates were verified by polymerase chain reaction (PCR) amplification of a specific region in the citrate synthase gene (*gltA*) using primers BhCS781.p and BhCS1137.n (Norman et al., 1995). Crude DNA extracts were obtained from isolates by heating a suspension of the microorganisms for 10 min at 95 °C. PCR amplifications were performed in a 25-μL reaction mixture containing 5 μL Green GoTaq PCR buffer (5×), 0.4 μmol of each primer, 200 μM each dNTP, 1 U Taq DNA polymerase (Promega, Madison, WI), and approximately 20 ng of template DNA. Positive and negative controls were included within each PCR assay to evaluate the presence of appropriately sized amplicons and contamination, respectively. Each PCR was carried out in a PTC 200 Peltier thermal cycler (MJ Research, Inc., MA) using the following program parameters: an initial 3-min step of denaturation at 95 °C followed by 35 cycles of 1 min denaturation at 95 °C, 1 min annealing at 55 °C, and 1 min elongation at 72 °C. Amplification was completed by holding the reaction mixture at 72 °C for 10 min. PCR products were identified by electrophoresis.

The *Bartonella* isolates were further examined by PCR for three additional genes: the cell division protein gene (*ftsZ*), the riboflavin synthase gene (*ribC*), and the RNA polymerase beta-subunit gene (*rpoB*), using primers described elsewhere (Renesto et al., 2001; Zeaiter et al., 2002; Johnson et al., 2003).

2.4. Sequencing and phylogenetic analysis of DNA

PCR positive products of the four examined genes were purified with the QIAquick PCR Purification Kit (Qiagen, Maryland) according to manufacturer's instructions and sequenced in both directions using an Applied Biosystems Model 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequencing reactions were carried out in a PTC 200 Peltier Thermal cycler using the same primers as the initial PCR assay at a concentration of 1.6 μM. Cycle parameters for the sequencing reactions were 45 cycles at 96 °C for 20 s, 50 °C for 20 s, and 60 °C for 4 min.

Sequences were analyzed using Lasergene (DNASTAR, Madison, WI) sequence analysis software to determine consensus of overlapping sequences for the amplified region of each gene. The Clustal V program within MegAlign (DNASTAR) was used to align and compare homology of *Bartonella gltA* sequences obtained from elk samples and from the GenBank database. The neighbor-joining method by Kimura's 2-parameter distance method and bootstrap calculation was carried out with 1000 resamplings. Novel sequences from the current study were submitted to GenBank and assigned unique accession numbers.

3. Results

3.1. Bartonella prevalence

We isolated *Bartonella* strains from four elk, and the identification of each isolate was confirmed by PCR and sequencing analyses of four genes – *gltA*, *ftsZ*, *ribC*, and *rpoB*. The overall prevalence of *Bartonella* in our sample of elk is 7.3% (4/55).

3.2. Phylogenetic analysis

The sequences obtained from characterization of the *Bartonella* isolates from elk were identical to each other by 4 target genes: *gltA*, *ftsZ*, *rpoB*, and *ribC* (GenBank accession numbers are HM167503, HM167504, HM167505, and HM167506, respectively). A phylogenetic tree based on *gltA* sequences is presented in Fig. 1 and demonstrates that the *Bartonella* isolates from elk clustered with *B. capreoli* with a similarity of 99.1%. Phylogenetic relationships also were analyzed by comparing sequences of *ftsZ*, *ribC*, and *rpoB* (data not shown). Analyses of both *ftsZ* and *rpoB* demonstrated that the elk-*Bartonella* clustered with *B. capreoli* with the similarities of 99.5% and 98.2%, respectively. Analysis of the *ribC* indicated that the elk- *Bartonella* had 98.6% similarity to *B. schoenbuchensis* and 98.4% similarity to *B. capreoli* and *B. chomelii*.

4. Discussion

Three of the four genetic markers analyzed indicated that the *Bartonella* isolates cultured from the elk in Wyoming were most closely related to *B. capreoli*. Although *ribC* is commonly used for DNA-based detection and characterization of *Bartonella* spp., our results suggested that the utility of this gene as a tool for phylogenetic analyses of *Bartonella* species is less evident (La Scola et al., 2003).

In the past, *B. capreoli* has been reported only from Europe following isolation from wild roe deer (Bermond

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