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PCR detection of *Bartonella bovis* and *Bartonella henselae* in the blood of beef cattle

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ABSTRACT

Although an organism primarily associated with non-clinical bacteremia in domestic cattle and wild ruminants, *Bartonella bovis* was recently defined as a cause of bovine endocarditis. The purpose of this study was to develop a *B. bovis* species-specific PCR assay that could be used to confirm the molecular prevalence of *Bartonella* spp. infection. Blood samples from 142 cattle were tested by conventional PCR targeting the *Bartonella* 16S–23S intergenic spacer (ITS) region. Overall, *Bartonella* DNA was detected in 82.4% (117/142) of the cattle using either *Bartonella* genus primers or *B. bovis* species-specific primers. Based upon size, 115 of the 117 *Bartonella* genus ITS PCR amplicons were consistent with *B. bovis* infection, which was confirmed by PCR using *B. bovis* species-specific primers and by sequencing three randomly selected, appropriately sized *Bartonella* genus PCR amplicons. By DNA sequencing, *Bartonella henselae* was confirmed as the two remaining amplicons, showing sequence similarity to *B. henselae* URBHLIE 9 (AF312496) and *B. henselae* Houston 1 (NC_005956), respectively. Following pre-enrichment blood culture of 12 samples in *Bartonella* alpha Proteobacteria growth medium (BAPGM) *B. henselae* infection was found in another three cows. Four of the five cows infected with *B. henselae* were co-infected with *B. bovis*. To our knowledge this study describes the first detection of *B. henselae* in any large ruminant species in the world and supports the need for further investigation of prevalence and pathogenic potential of *B. henselae* and *B. bovis* in cattle.

1. Introduction

To date, at least 22 species or subspecies of the genus *Bartonella* have been characterized, however, only two species, *Bartonella bovis* (previously described as *Bartonella weissii*) and *Bartonella chomelii*, have been found to infect cattle (Chang et al., 2000; Breitschwerdt et al., 2001; Maillard et al., 2004; Kelly et al., 2005). *Bartonellae* are small intracellular, gram-negative bacteria, and all species are thought to be transmitted by blood-sucking arthropods (Maurin and Raoult, 1996; Chomel, 2000; Chang et al., 2001; Halos et al., 2004; Alexander, 2005; Skotarczak and Adamska, 2005). In 2004, *Bartonella* DNA was amplified for the first time in North American flies collected from beef and dairy cattle barns and feedlots in California (Chung et al., 2004). In that study, a sequence identical to *B. bovis* was obtained from two horn flies (*Haematobia* spp.) collected from a beef cattle barn. A definite route of transmission of *B. bovis* to cattle has yet to be determined, but it has been suggested that ticks may be a potential vector (Chang et al., 2001; Skotarczak and Adamska, 2005). The detection of *Bartonella* spp. in biting flies and ticks, common ectoparasites of ruminants, supports their potential role for *B. bovis* transmission to cattle (Chung et al., 2004; Halos et al., 2004).

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Detection of *Bartonella* spp. infection in beef cattle is of potential importance because these highly prevalent and previously unknown intravascular bacteria may play an unrecognized role in complex disease expression (Merrell and Falkow, 2004). Although an organism primarily associated with non-clinical bacteremia in domestic cattle and wild ruminants in North America (Chang et al., 2000; Breitschwerdt et al., 2001; Maillard et al., 2004), domestic cattle in Europe (Bermond et al., 2002; Maillard et al., 2004, 2006) and in Africa (Kelly et al., 2005), *B. bovis* was recently defined as a cause of bovine endocarditis (Maillard et al., 2007). The extent to which *B. bovis* may interact with or complicate the diagnosis of other infectious and non-infectious diseases of cattle is unknown. The high prevalence of *B. bovis* infection found in many cattle populations studied to date indicates that this organism shares a strong evolutionary adaptation with ruminant species; however, it is possible that chronic infection with these intravascular bacteria contributes to pathology during states of stress, nutritional deprivation or exposure to toxins (Breitschwerdt and Kordick, 2000).

In this study, *B. bovis* infection was identified in 82.4% of North Carolina beef cattle. Using IFA serology, a previous study from North Carolina found 36 of 38 cows had antibodies (>1:32) to *B. bovis* (*B. weissii*) antigens (Breitschwerdt et al., 2001). In a study from California, *Bartonella* bacteremia was documented by blood culture isolation in 81–96% of beef cattle (Chang et al., 2000). In that same study, only 17% of the dairy cattle population from the same region was found to be *Bartonella* bacteremic. In contrast to the California results, our laboratory has experienced difficulty in obtaining *B. bovis* isolates by either direct plating (Breitschwerdt et al., 2001) or by using BAPGM enrichment culture of stored-frozen samples as described in this study. The reason(s) for this discrepancy remains unclear.

A rapid, efficient, and cost effective means to detect *Bartonella* infection in ruminants would facilitate future clinical, pathological and epidemiological studies. Therefore, the purpose of this study was to generate *B. bovis*-specific ITS primers, to compare the sensitivity of these primers to *Bartonella*-genus-specific primers, and to determine the molecular prevalence of *Bartonella* spp. infection in several beef cattle herds. The 16S–23S intergenic spacer region was targeted because this PCR assay provides sensitive and specific detection of *Bartonella* spp. DNA in biological samples (Roux and Raoult, 1995; Minnick and Barbian, 1997; Jensen et al., 2000; Houpikian and Raoult, 2001; Maillard et al., 2004; García-Esteban et al., 2008). In conjunction with this study, co-infection with *B. henselae* and *B. bovis* was found in four cows and one cow was infected solely with *B. henselae*, which represents the first report of infection with this *Bartonella* sp. in ruminants.

2. Materials and methods

2.1. Study population

From February to November 2001, 142 EDTA anti-coagulated blood samples were collected from three separate beef cattle herds located in the Piedmont region of North Carolina, specifically Durham, Wake and Sampson counties. There were no specific exclusion criteria that limited entry into the study, therefore age, sex, breed, health status, and farm management practices were not controlled.

2.2. DNA extraction

DNA was extracted from 200 μl of frozen EDTA-blood samples using a previously described extraction technique (Diniz et al., 2007).

2.3. Bartonella ITS (16S–23S ribosomal RNA intergenic spacer) genus PCR amplification

Bartonella genus screening was performed as previously described (Maggi and Breitschwerdt, 2005a). PCR screening of *Bartonella* ITS region was performed in all 142 samples using oligonucleotides 325 s: 5′-CTT CAG ATG ATG ATC CCA AGC CTT CTG GCG-3′ and 1100as: 5′-GAA CCG ACG ACC CCC TGC TTG CAA AGC A-3′ as forward and reverse primers, respectively. Amplification was performed in a 25 μl final volume reaction containing 12.5 μl of Tak-Ex® Premix (Fisher Scientific), 0.25 μl of 30 μM of each forward and reverse primer (IDT® DNA Technology), 8 μl of molecular-grade water, and 5 μl of DNA from each sample were tested. PCR negative controls were prepared using 5 μl of DNA from blood of a healthy, specific pathogen-free dog. Positive controls for PCR were prepared by serial dilution (using dog blood DNA) of *Bartonella* genomic DNA (down to 0.002 pg/μl stock) from *B. henselae*. PCR was performed in an Eppendorf Mastercycler EPgradient® under the following conditions: a single hot-start cycle at 95 °C for 2 min followed by 55 cycles of denaturing at 94 °C for 15 s, annealing at 66 °C for 15 s, and extension at 72 °C for 18 s. Amplification was completed by an additional cycle at 72 °C for 1 min, and products were analyzed by 2% agarose gel electrophoresis with detection using ethidium bromide under ultraviolet light.

2.4. Bartonella bovis ITS (16S–23S ribosomal RNA intergenic spacer) PCR amplification

All 142 samples were also tested using newly designed *B. bovis* species-specific primers that incorporated the same PCR conditions described above and the same reverse primer, but the new assay incorporated a forward primer that is specific for the amplification of *B. bovis* (*B. bovisf*: 5′-GGA GCG TTT AAA AAA ACA AAC CAA AAG CG-3′).

2.5. Bartonella species pre-enrichment culture

One milliliter of blood, previously frozen for several years, from 10 *B. bovis* and the 2 *B. henselae* PCR positive cattle were inoculated into liquid *Bartonella* alpha-Proteobacteria growth medium (BAPGM) and incubated at 35 °C in 5% CO_2, water-saturated atmosphere as previously described (Maggi et al., 2005b; Maggi and Breitschwerdt, 2005c; Duncan et al., 2007). Following a 7-day incubation period, a 1 ml sample from the liquid