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Evaluation of conventional and real-time PCR assays for detection and differentiation of Spotted Fever Group *Rickettsia* in dog blood

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Abstract

Spotted Fever Group *Rickettsia* is important cause of emerging and re-emerging infectious disease in people and dogs. Importantly, dogs can serve as sentinels for disease in people. Sensitive and specific diagnostic tests that differentiate among species of infecting *Rickettsia* are needed. The objective of this study was to develop a sensitive and specific PCR that differentiates SFG *Rickettsia* infecting dog blood. Conventional and real-time PCR assays were developed using primers that targeted a small region of the *ompA* gene. Their sensitivity, determined by testing a cloned target sequence in the presence of host DNA, was 15–30 and 5 copies of DNA, respectively. Testing of *Rickettsia* cultures and analysis of *Rickettsia* gene sequences deposited in GenBank verified DNA could be amplified and used to differentiate species. DNA from the blood of infected dogs was also tested. Importantly, *Rickettsia* DNA was detected before seroconversion in some dogs. The species of infecting *Rickettsia* was also identified. We conclude these assays may assist in the timely diagnosis of infection with SFG *Rickettsia*. They may also facilitate the discovery of novel SFG *Rickettsia* infecting dogs, and in the investigation of dogs as sentinels for emerging rickettsioses.

Keywords: Dog; PCR; *Rickettsia*; Spotted fever

1. Introduction

Spotted Fever Group (SFG) *Rickettsia* infect dogs and people, and are important causes of emerging infectious disease (Parola et al., 2005; Raoult and Roux, 1997; Breitschwerdt et al., 1988). Dogs develop spotted fever-type illness and may serve as sentinels for human infection with *Rickettsia rickettsii*, the cause of Rocky Mountain spotted fever (RMSF) and *Rickettsia conorii*, the cause of Mediterranean spotted fever (MSF) (Elchos and Goddard, 2003; Paddock et al., 2002; Mannelli et al., 2003; Solano-Gallego et al., 2006). Whether dogs develop disease from, and

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are sentinels for, other SFG rickettsioses are not yet known.

Although well characterized, clinical signs of spotted fever rickettsioses are not specific and can occur in the absence of history of a tick bite in both dogs and people. Thus diagnosis can be challenging. The consequences of delayed or misdiagnosis are severe morbidity and death (Gasser et al., 2001; Abrahamian, 2000).

Importantly, currently used diagnostic tests have limitations. Serological titers can be negative acutely, a time when rapid diagnosis is critical, and may persist after infection or exposure (Breitschwerdt et al., 1990; Breitschwerdt et al., 1988; Parola et al., 2005; Tesouro et al., 1998). *Rickettsia* spp. circulate in blood in low numbers during the acute phase of infection (Parola et al., 2005; Breitschwerdt et al., 1990). Therefore, PCR may be useful in detecting infection prior to seroconversion, and help differentiate exposure from active infection. However, the sensitivity of PCR assays that detect *Rickettsia* infection in dogs has not been determined. Furthermore, routine serologic testing and PCR assays used to detect infection in dogs do not differentiate among rickettsial species (Hechemy et al., 1989; La Scola and Raoult, 1997; Breitschwerdt et al., 1999; Breitschwerdt et al., 1988).

The rickettsial *ompA* gene is an attractive target for detecting and differentiating SFG *Rickettsia* using PCR because this well characterized gene exhibits significant variability among species (Fournier et al., 2003; Roux et al., 1996). In addition, its protein product is an outer membrane protein that contributes to serologic differences used for phenotypic species classification (Xu and Raoult, 1997). Therefore sequencing this gene provides a rational link between phenotypic and genotypic species differentiation. Sequence information from a hypervariable 632 base pair gene product amplified using primers RR.190.70F and RR.190.701R has been used to determine the species of *Rickettsia* infecting human patients (Giammanco et al., 2005a,b; Raoult and Roux, 1997). The limit of detection for a PCR using these primers was shown to be 160 copies of SFG *Rickettsia* (Fournier and Raoult, 2004). However, the calculation for the limit of detection was not performed in the presence of host DNA, and thus sensitivity is likely to be lower in clinical use.

The purpose of this study was to design a sensitive and specific PCR that could detect and differentiate species of SFG *Rickettsia* present in dog blood by targeting a small portion of the 632 base pair hypervariable region of the *ompA* gene, and to evaluate the diagnostic utility of this assay in infected dogs.

2. Materials and methods

2.1. Bacterial strains

Cell cultures of *Rickettsia canadensis* McKiel LAA1, *Rickettsia montana* OSU 85-930, *Rickettsia parkeri* HmacA, *Rickettsia prowazekii* F-16 strain (flying squirrel isolate) L3B1, *R. prowazekii* strain Breinl, *Rickettsia rhipicephali* CA871 V4P13, *R. rickettsii* strain HLP#2, *R. rickettsii* strain Bitterroot, and *Rickettsia typhi* strain RTW were kindly provided by Dr. Gregory Dasch. *Rickettsia amblyommi* WB8.2, *R. conorii* Israel 2, *R. conorii* Mor VR141, *R. rickettsii* strain Sheila Smith, were kindly provided by Dr. David Walker. *Rickettsia* cultures were stored at −70 °C until analysis.

2.2. Serology

Immunofluorescence testing was performed as previously described (Kordick et al., 1999).

2.3. DNA template

DNA was extracted from *Rickettsia* cell cultures using the QIAamp® DNA blood mini kit (Qiagen Inc., Valencia, CA) according to manufacturer's instructions. DNA from dogs naturally infected with *R. rickettsii* was extracted from banked frozen whole blood in EDTA using the QIAamp® DNA blood, or the BIOROBOT M48 DNA Robot using the Qiagen MagAttract® DNA extraction kit (Qiagen Inc., Valencia, CA). DNA was extracted from 200 μl of blood of dogs naturally infected with *R. conorii* as described (Solano-Gallego et al., 2006). Genomic DNA was quantitated using the Nanodrop ND1000 Spectrophotometer® and Genom-48 Version 2® software (NanoDrop Technologies, Wilmington, DE). The integrity of the DNA extracted from the blood of dogs was tested using a PCR that amplifies the glyceraldehyde-3-phosphate dehydrogenase pseu-