Anaplasma platys in Dogs, Chile

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We conducted a 16S rRNA nested PCR for the genus *Ehrlichia* and *Ehrlichia* spp. with blood samples from 30 ill dogs in Chile. Phylogenetic analysis was performed by using *groESL* gene amplification. We identified *Anaplasma platys* as 1 of the etiologic agents of canine ehrlichiosis.

*Ehrlichias* are recognized as important emerging tick-borne diseases in humans and wild and domestic animals. The brown dog tick, *Rhipicephalus sanguineus*, is the main tick that infests dogs in Chile (1). This tick species is a vector of *Ehrlichia canis* and has been implicated, but not confirmed, as a vector of *Anaplasma platys* (2). Serologic and clinical evidence of canine ehrlichiosis and serologic evidence of human ehrlichiosis have been reported in Chile (3,4). The purpose of this study was to identify the etiologic agent of canine ehrlichiosis in Chile.

The Study

Blood samples were obtained from 30 pet dogs seen in a private veterinary clinic in Santiago, Chile, with tick infestation and clinical signs compatible with ehrlichiosis (hemorrhagic manifestations and thrombocytopenia). We performed a nested PCR to amplify a portion of the 16S rRNA gene by using specific primers for the genus *Ehrlichia* and for *Ehrlichia* spp. DNA was extracted from 300 μL of whole blood by using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA). For *Ehrlichia* genus–specific PCR, 2.5 μL of DNA was amplified by using outer primers EHR-OUT1 and EHR-OUT2 and inner primers GE2F and EHR3-IP2 in 1 reaction with a final volume of 25 μL (5) (Table 1). The first-round amplification included 20 cycles of denaturation at 94°C for 45 s, annealing at 72°C for 1.5 min, and chain extension at 72°C for 1.5 min. The second-round amplification included 50 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 1 min, and chain extension at 72°C for 1 min, followed by a final extension at 72°C for 5 min. Amplification products were analyzed by agarose gel electrophoresis. The expected size of the amplification product was 120 bp. *A. platys* 16S rRNA DNA was used as a positive control (provided by Didier Raoult). For *Ehrlichia* spp.–specific amplification, we used the same set of outer primers for *Anaplasmataceae* and specific inner primers for *A. phagocytophilum* (6), *E. chaffeensis*, *E. ewingii*, and *E. canis* (5) (Table 1). For *A. platys* amplification, we used inner primers developed by Kordick et al. (EHR3-IP2–*E. platys*) (7) (Table 1). Expected sizes of amplification products were 546, 395, 395, 389, and 151 bp, respectively.

The *Ehrlichia* genus PCR resulted in the expected DNA band in 6 of 30 dogs (dogs 7, 12, 17, 19, 23, and 25). These 6 samples were positive only for *A. platys*, showing the expected 151-bp product, and negative for other species tested (Figure 1, panel A). *A. platys* PCR was also conducted on the remaining 24 *Ehrlichia*-negative samples; none were positive.

DNA obtained from 3 16S rRNA PCR products (dogs 7, 17, and 25) was purified by using a commercial kit (Rapid Gel Extraction System; Marligen Biosciences, Ljamsville, Germany) and sequenced twice with an ABI 3100 genetic analyzer (Model 3100; Applied Biosystems, Foster City, CA, USA). The 16S rRNA sequences obtained were compared by using BLAST (www.ncbi.nlm.nih.gov/blast) with sequences available at GenBank. Sequences obtained were similar to that of *A. platys* strain Okinawa 1 (GenBank accession no. AF536828), with similarities of 98%, 95%, and 98%, respectively. GenBank accession nos. for 16S rRNA sequences of *A. platys* strains obtained in this study are DQ125260 and DQ125261, which correspond to strains from dogs 7 and 17, respectively.

For phylogenetic analysis, the *groESL* gene of *A. platys* was amplified from samples positive for *A. platys* 16S rRNA that had sufficient amounts of DNA (dogs 7, 17, and 25) and 1 negative sample (dog 13). Reactions contained 2 μL of purified DNA as template in a total volume of 25 μL. Amplifications contained 1.25 U *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA), 3 mmol/L MgCl₂, 2.5 mmol/L deoxynucleotide triphosphates (Invitrogen), and 0.2 pmol/L of primers EEgro1F and EEgro2R (8) (Table 1). DNA was denatured by heating at 95°C for 10 min. PCR amplification included 40 cycles of denaturation at 95°C for 1.5 min, annealing at 52°C for 2 min, and extension at 72°C for 1.5 min, followed by a final extension at 72°C for 10 min. For nested amplifications, 1 μL of primary PCR products was used as the template in a total volume of 25 μL. Reaction conditions were the same as for primary amplifications. The primers used were SQ3F, SQ5F, SQ4R, and SQ6R (9) (Table 1). PCR products were analyzed by 1.5% agarose gel electrophoresis.

We amplified 3 overlapping fragments (790, 1,170, and 360 bp) in 3 16S rRNA–positive samples (Figure 1, panel B). These DNAs were purified by using a commercial kit (Rapid Gel Extraction System; Marligen), sequenced, and analyzed for phylogenetic relationships. Multiple alignment
A wide range of clinical manifestations of canine cyclic thrombocytopenia has been described. Cases from the United States have been described as mild or asymptomatic (10), and cases from Spain have more severe symptoms (11), which also seems to be the case in Chile. This variability in clinical symptoms of infection has not been clearly associated with strain variations (11–13).

Low diversity was observed when groESL gene sequences of Chilean strains were compared with other A. platys strains available in GenBank. This finding has also been observed in strains from different geographic origins (13).

Conclusions

We identified A. platys DNA in the blood of 6 dogs with clinical signs indicative of ehrlichiosis. These findings support the conclusion that A. platys is an etiologic agent of canine ehrlichiosis in Chile.

Since its first report in the United States in 1978 (10), A. platys has been described in several countries as the etiologic agent of cyclic thrombocytopenia in dogs. A tick vector of A. platys has not been determined, although R. sanguineus is the most suspected species (2). Because R. sanguineus is the only tick species that infests dogs in Santiago (1), our results support the conclusion that this species is the vector of A. platys in Chile.
Recent studies have shown more genetic variability when sequences of the \textit{gltA} gene were used (11,12). Evidence of the zoonotic potential of \textit{A. platys} is scarce. In Venezuela, a few symptomatic human cases have been diagnosed since 1992 by the presence of platelet morulae in blood smears (14). Monocytic and platelet morulae were reported in a 17-month-old girl with fever and rash (15). However, none of these cases have been confirmed by molecular assays. Further studies that investigate the pathogenic and zoonotic role of \textit{A. platys} should be conducted.

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References


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