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Molecular detection and characterization of *Anaplasma platys* in dogs and ticks in Cuba

Claudia Bezerra da Silva[a,*], Huarrisson Azevedo Santos[b], Maylín González Navarrete[c], Carla Carolina Dias Uzedo Ribeiro[a], Belkis Corona Gonzalez[d], Maykelin Fuentes Zaldivar[d], Marcus Sandes Pires[a], Maristela Peckle[a], Renata Lins da Costa[a], Gabriela Lopes Vivas Vitari[a], Carlos Luiz Massard[a]

[a] *Department of Animal Parasitology, Veterinary Institute, Federal Rural University of Rio de Janeiro, Br 465, km 7, Seropedica, Rio de Janeiro, 23897-000, Brazil*
[b] *Department of Epidemiology and Public Health, Veterinary Institute, Federal Rural University of Rio de Janeiro, Br 465, km 7, Seropedica, Rio de Janeiro, 23897-000, Brazil*
[c] *Department of Animal Prevention, Veterinary Medicine College, Agrarian University of Habana, Mayabeque, Cuba*
[d] *Department of Microbiology, National Center for Animal and Plant Health, Mayabeque, Cuba*

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ABSTRACT

Canine cyclic thrombocytopenia, an infectious disease caused by *Anaplasma platys* is a worldwide dog health problem. This study aimed to detect and characterize *A. platys* deoxyribonucleic acid (DNA) in dogs and ticks from Cuba using molecular methods. The study was conducted in four cities of Cuba (Habana del Este, Boyeros, Cotorro and San José de las Lajas). Blood samples were collected from 100 dogs in these cities. The animals were inspected for the detection of tick infestation and specimens were collected. Genomic DNA was extracted from dog blood and ticks using a commercial kit. Genomic DNA samples from blood and ticks were tested by a nested polymerase chain reaction (nPCR) to amplify 678 base pairs (bp) from the 16S ribosomal DNA (*rDNA*) of *A. platys*. Positive samples in nPCR were also subjected to PCR to amplify a fragment of 580 bp from the citrate synthase (*gltA*) gene and the products were sequenced. Only *Rhipicephalus sanguineus* sensu lato (s.l.) was found on dogs, and 8.93% (n = 5/56) of these ticks plus sixteen percent (16.0%, n = 16/100) of dogs were considered positive for *A. platys* by nPCR targeting the 16S *rDNA* gene. All analyzed *gltA* and 16S *rDNA* sequences showed a 99–100% identity with sequences of *A. platys* reported in around the world. Phylogenetic analysis showed two defined clusters for the 16S *rDNA* gene and three defined clusters for the *gltA* gene. Based on the *gltA* gene, the deduced amino acid sequence showed two mutations at positions 88 and 168 compared with the sequence DQ525687 (GenBank ID from Italian sample), used as a reference in the alignment. A preliminary study on the epidemiological aspects associated with infection by *A. platys* showed no statistical association with the variables studied ($p > 0.05$). This is the first evidence of the presence of *A. platys* in dogs and ticks in Cuba. Further studies are needed to evaluate the epidemiological aspects of *A. platys* infection in Cuban dogs.

1. Introduction

Anaplasma platys is an obligatory intracellular Gram-negative bacterium (French and Harvey, 1983; Harvey et al., 1978). The reclassification of *Ehrlichia platys* as *A. platys* was based on a phylogenetic study of the 16S *rDNA* gene (Dumler et al., 2001). This microorganism causes infectious canine cyclic thrombocytopenia (ICCT) due to the cyclic occurrence of parasitemia in platelets (Harrus et al., 1997). A recent study demonstrated that, although this rickettsial pathogen has tropism for platelets, it can also infect promegakaryocytes and megakaryocytes, which are the precursor cells from bone marrow (De Tommasi et al., 2014).

The etiological agent of ICCT enters the cell by adhesion to the surface followed by endocytosis, and the vacuolar membrane is probably derived from the outer membrane of platelets (Harvey, 2011). Repeated binary fission of the organism within the vacuole results in the formation of a morula (Harvey et al., 1978) causing direct injury to platelets. The main clinically observable

* Corresponding author.
E-mail address: claudia_ufrrj@yahoo.com.br (C.B.d. Silva).

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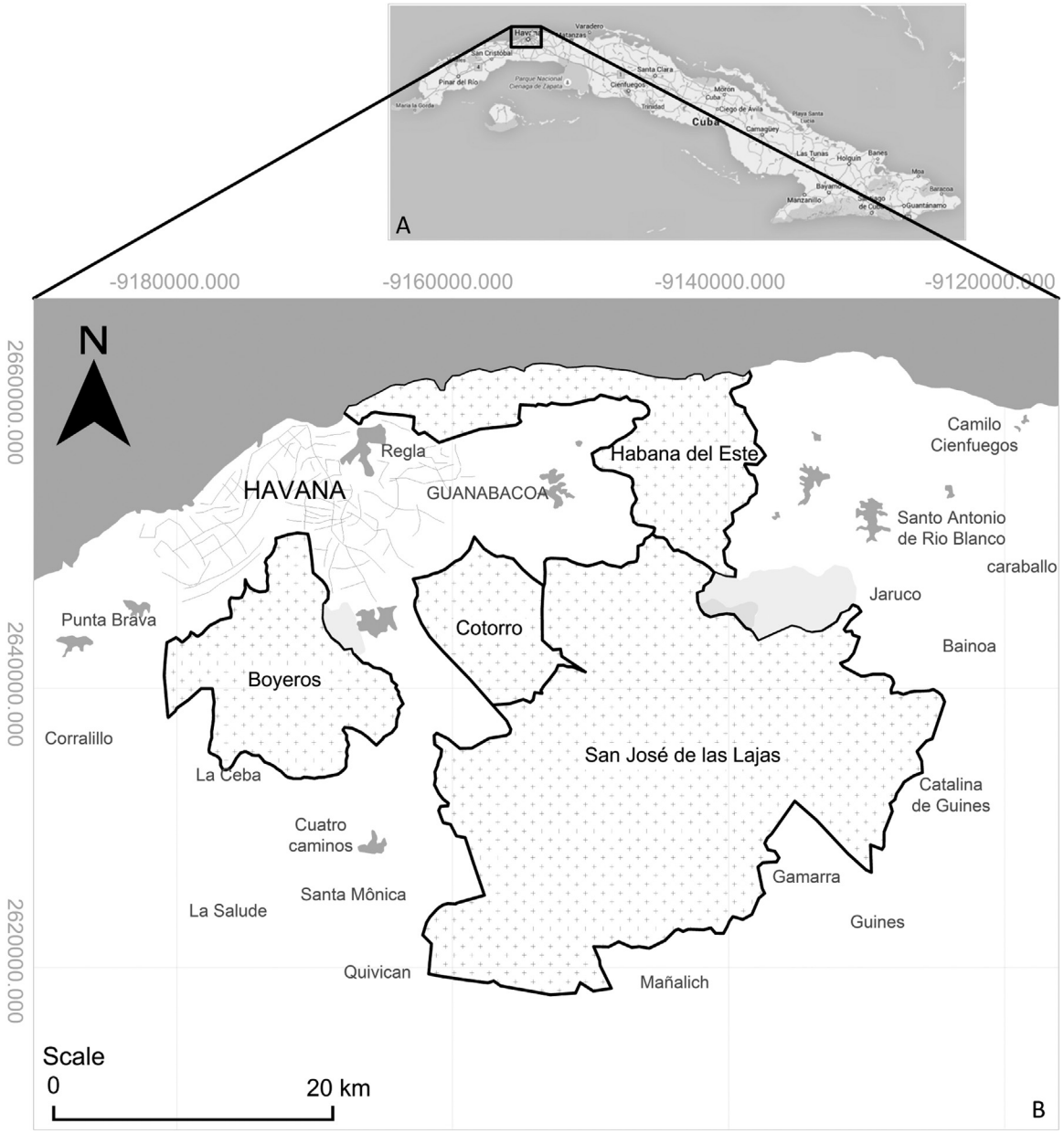

Fig. 1. A: Island of Cuba. B: location of the cities in which the collections were taken in the provinces of Habana (Cotorro, Boyeros and Habana del Este) and Mayabeque (San José de las Lajas). Scale bar = 20 Km.

symptoms of this disease are depression, fever and anorexia, but asymptomatic infections may occur (Harvey et al., 1978; Harvey, 2011). Ecchymotic hemorrhages were reported in a case of *A. platys* infection in Greece (Kontos et al., 1991). Thrombocytopenia, anemia (Dagnone et al., 2003), monocytosis, and presence of macroplatelets (French and Harvey, 1983; Harrus et al., 1997; Antognoni et al., 2014) are the most common findings observed in the laboratory.

Anaplasma platys is likely transmitted to dogs by the tick *Rhipicephalus sanguineus* sensu lato (s.l.) (Sanogo et al., 2003; Yabsley et al., 2008) although their role in transmission has not been proven experimentally (Simpson et al., 1991). This tick species is widely distributed around the world and infests primarily domestic dogs, supporting its role as a vector of several important pathogens (Yabsley et al., 2008). However, *A. platys* DNA was also detected in *Dermacentor auratus,* which infests dogs in Thailand (Parola et al., 2013), but the actual role of this tick in the transmission of this bacterium has not been stablished. Although dogs are considered the principal host for *A. platys*, other studies have detected this agent in domestic cats (Correa et al., 2011), cows (Dahmani et al., 2015a) and humans (Arraga-Alvarado et al., 1999, 2014; Breitschwerdt et al., 2014).

In regard to the geographic distribution of *A. platys*, several studies have reported the occurrence of seropositive dogs in different areas in the United States (French and Harvey, 1983), Greece (Kontos et al., 1991) and Belgium (Heyman et al., 2007). The molecular detection of *A. platys* in dogs was reported in countries in different continents, including South America (Abarca et al., 2007; Dahmani et al., 2015b; Ferreira et al., 2007), Central America (Rojas et al., 2014); (Santamaria et al., 2014), North America (Kordick et al., 1999; Almazán et al., 2016), Europe (Dumler et al., 2001; Inokuma et al., 2002a), Asia (Inokuma et al., 2002b; Unver et al., 2003), Oceania (Brown et al., 2001) and Africa (Dahmani et al., 2015c; Sanogo et al., 2003).

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