Case Report: Molecular Evidence of Anaplasma platys Infection in Two Women from Venezuela

Cruz M. Arraga-Alvarado, Barbara A. Qurollo, Omaire C. Parra, Maribel A. Berrueta, Barbara C. Hegarty, and Edward B. Breitschwerdt*

Unidad de Investigaciones Clínicas, Facultad de Ciencias Veterinarias, Universidad del Zulia, Maracaibo, Venezuela; Intracellular Pathogens Research Laboratory, Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

Abstract. This article presents two case reports of Anaplasma platys detection in two women from Venezuela. Both patients were exposed to Rhipicephalus sanguineus, the presumed tick vector, and experienced chronic, nonspecific clinical signs including headaches and muscle pains. Intra-platelet inclusion bodies resembling A. platys were observed in buffy coat smears and A. platys DNA was amplified and sequenced from whole blood; however, treatment with doxycycline did not alleviate their symptoms. These cases provide further support for A. platys as a zoonotic tick-borne pathogen, most likely of low pathogenicity; nonetheless, the cause of illness in humans by A. platys is yet to be confirmed.

INTRODUCTION

Microorganisms invading platelets have been observed in people from Maracaibo, Venezuela since 1992, associated with clinical symptoms such as fever, chills, headache, muscular pain, arthralgia, weakness, insomnia, skin lesions, and other symptoms similar to those found in human Ehrlichiosis and Anaplasmosis.1-2 Between 1993 and 2012, a total of 5,954 people had intra-platelet inclusions in buffy coat smear (BCS) evaluated at the Unidad de Investigaciones Clínicas-Facultad de Ciencias Veterinarias-La Universidad del Zulia (UIC-FCV-LUZ). Most had moderate to severe clinical symptoms, some were hospitalized, and all required treatment to recover health. Some patients responded well to tetracyclines, especially to doxycycline. When platelet-rich plasma from BCS-positive cases was prepared for ultrastructural examination by transmission electron microscopy, and organisms compared with ultrastructural studies described in the United States3 and in Venezuela,4 it was concluded that organisms infecting dogs and people appeared different. In canine organisms, a well-defined double membrane, characteristic of the Anaplasmataceae family, was evident and the intra-vacuolar space was clear, whereas in organisms from human cases, organism membranes were thickened and the intra-vacuolar space appeared electron-dense.5 To date, the etiology of these intra-platelet organisms has not been identified.

Case 1. A 29-year-old woman from Maracaibo, Zulia State, Venezuela resided in a household consisting of three other people, three dogs, and two cats. In February 2012, the woman removed a tick from her chest and leg. During the second week of March she developed decreased appetite, generalized weakness, muscular pain, fatigue, and occasional headaches, all of which persisted during the next 2 months. On March 30, 2012, an EDTA-anti-coagulated blood specimen was sent to the UIC-FCV-LUZ. A BCS was prepared, stained with a Wright’s Giemsa (Dip Quick) stain and examined by light microscopy at high magnification (100×). Intra-platelet inclusions, morphologically consistent with Anaplasma platys, were visualized. Complete blood count (CBC) values, obtained the same day, were within reference ranges, red blood cell (RBC) 4.48 × 10¹²/L (3.5–5.0 × 10¹²/L), Hct 44% (36–48%), Hgb 14.4g/dL (11–15g/dL), white blood cell (WBC) 6.7 × 10⁹/L (4.3–10.8 × 10⁹/L), with segmented neutrophils 4.69 × 10⁹/L (3.0–5.0 × 10⁹/L), lymphocytes 1.80 × 10⁹/L (1.0–3.0 × 10⁹/L), and eosinophils 0.223 × 10⁹/L (0.02–0.35 × 10⁹/L); platelet count was 311 × 10⁹/L (150–400 × 10⁹/L). A blood sample was obtained for DNA extraction, after which DNA was stored at −20°C for future polymerase chain reaction (PCR) testing to detect potential intracellular rickettsial pathogens. A second blood sample, obtained in April 2012, for BCS, also contained intra-platelet inclusions (Figure 1). Subsequently, a physician prescribed Acetaminophen, 500 mg every 8 hours until the symptoms disappeared, and Vitamin C, 500 mg daily for 7 days. Muscle pain and headaches resolved; however, occasionally during 2012 the woman experienced flu-like symptoms including nasal discharge, weakness, and headaches. The PCR testing providing molecular evidence supporting A. platys infection in the March 30 blood sample was not completed until August 2013. After this result became available, repeat blood smear examination again identified intra-platelet inclusions similar to A. platys, after which her physician prescribed doxycycline for treatment of the A. platys infection.

Concurrently, during February 2012 when the woman experienced tick attachments, a heavy infestation of Rhipicephalus sanguineus (R. sanguineus) ticks was found on the three adult dogs living in the patient’s house. Petechial lesions were visible on the abdomen, thorax, and legs of two of the three dogs. The CBCs from the two dogs with clinical signs documented thrombocytopenia, 20.0 × 10⁹/L and 43.0 × 10⁹/L, respectively (reference range 175–500 × 10⁹/L). No organisms were visualized on blood smears. Using a commercially available ELISA kit, SNAP4DX, (IDEXX Laboratories Inc., Westbrook, ME) that employs synthetic peptides of Ehrlichia spp., Anaplasma spp., Borrelia burgdorferi (Lyme Disease), and Dirofilaria immitis (Heartworm Disease) antigen, both thrombocytopenic dogs were only Anaplasma spp. seroreactive, most likely indicative of prior or concurrent infection with A. platys, an Anaplasma sp. known to be endemic to Venezuela. Both dogs were promptly treated with an acaricide to kill the ticks and doxycycline, 10 mg/kg, every 12 hours, was administered for 21 consecutive days with resultant clinical and hematological recovery, as indicated by normalization of platelet counts.

*Address correspondence to Edward B. Breitschwerdt, 1060 William Moore Dr., Raleigh, NC 27607. E-mail: ed_breitschwerdt@ncsu.edu
isms were visualized. During 2012 Treatment was not prescribed at the time intra-platelet organ-

+ (segmented neutrophils 1.692 (311 + (1.836

that experienced recurrent 

and insomnia. In addition, she had recently obtained a dog 

woman reported muscular pain, arthralgia, headaches, chills, 

such as anaplasmosis and ehrlichiosis. 

the presence of morulae, associated with tick-borne diseases, 

Her doctor had requested the BCS examination to rule out 

however, the woman was never knowingly bitten by a tick. 

Rickettsia typhi 

DNA was 

-°

ewtions. Extracted DNA samples were stored at 

20°C until use. The DNA integrity was verified in 1% agarose gels. 

PCR. DNA from both cases was tested by PCR assays that 

amply Anaplasma and Ehrlichia sp. PCR. The DNA was 

obtained from 2.5 mL of whole blood from each using a 

BDtract Genomic DNA Isolation Kit (Maxim Biotech, Inc., 

San Francisco, CA), according to manufacturer’s recommenda-

tions. Extracted DNA samples were stored at −20°C until use. 

DNA extraction. Fresh blood samples were collected 

for Anaplasma and Ehrlichia sp. DNA of the following 

genes: Ehrlichia/Anaplasma 16S ribosomal RNA (rRNA), 

A. platys p44, E. canis p30 (msp4), and A. phagocytophilum 
p44. All PCRs were performed in an Eppendorf Mastercycler 

EPgradient (Eppendorf, Hauppauge, NY) with an aluminum 

block and included positive (plasmid or culture genomic 

DNA) and negative (RNAse-free, molecular grade water 

blocks and included positive (plasmid or culture genomic 

DNA) controls for each assay. 

The PCR product visualization was performed using the 

horizontal electrophoresis system Wide Mini-Sub Cell GT 

(Bio-Rad, Hercules, CA) and AlphaImager 3300 visualization computer software 

for Windows 2000/XP (ProteinSimple, Santa Clara, CA) and AlphaImager 3300 visualization computer software 

for Windows 2000/XP (ProteinSimple, Santa Clara, CA). Sequences were obtained by direct submission to GENEWIZ, 

Inc. (Research Triangle Park, NC). Alignments were compared 

with GenBank sequences using AlignX software (Vector NTI 


Ehrlichia and Anaplasma 16S rRNA PCR. Oligonucleo-

tides and PCR conditions designed to amplify a 420 base 

pair (bp) fragment of the 16S rRNA gene have been described previously.3 

Anaplasma platys p44 PCR. The PCR screening for the A. 

platys p44 gene was performed using Apl_p44F3 (5′-GCT 

AAG TGG AGC GGT GGC GAT GAC AG) and 

Apl_p44R3 (5′-CGATCTCCGCCGCT TTTGATTTCTTC) as 

forward and reverse primers, respectively. Amplification, 

which yields a 520 bp amplicon, was performed in a 25 μL 

final volume reaction containing 12.5 μL of MyTaq HS 

Mix (2×) (Bioline cat: BIO-25046), 0.3 μL of 50 mM of each 

primer (Sigma-Aldrich, St. Louis, MO), 7 μL of filter-sterilized,
Aph_p44_F (5'-GAAATCTGGGACTGTTGTATCCTTG) and Aph_p44_R (5'-GAAATGTAGCTTCGTTATTGGA) as forward and reverse primers, respectively. Amplification, as in glyc cycle at 72°C for 1 minute.

Ehrlichia canis p30 (msp4) PCR. The PCR screening for the E. canis p30 gene was performed using Ec_msp4_F2 (5'-GAATCATGGGACTGTTGTATCCTTG) and Ec_msp4_R (5'-GAAATGTAGCTTCGTTATTGGA) as forward and reverse primers, respectively. Amplification, which yields a 470 bp amplicon, was performed in a 25 μL final volume reaction containing 12.5 μL of MyTaq HS Mix (2×) (Bioline cat: BIO-25046), 0.25 μL of 50 μM of each primer (Sigma-Aldrich), 7 μL of filter-sterilized, molecular-grade water and 5 μL of extracted DNA template. The PCR was performed using a single hot start cycle at 94°C for 3 minutes, followed by 55 cycles of denaturation at 94°C for 15 s, annealing at 62°C for 15 s, and extension at 72°C for 15 s, followed by a single cycle at 72°C for 1 minute.

Anaplasma phagocytophilum p44 PCR. The PCR screening for the A. phagocytophilum p44 gene was performed using Aph_p44_F (5'-CTGAGGAAATGTGTTAGGGCCTCA) and Aph_p44_R (5'-CCCAAATCCGGAGGCTAGGTTGG) as forward and reverse primers, respectively. Amplification, which yields a 230 bp amplicon, was performed in a 25 μL final volume reaction containing 12.5 μL of MyTaq HS Mix (2×) (Bioline cat: BIO-25046), 0.25 μL of 50 μM of each primer (Sigma-Aldrich), 7 μL of filter-sterilized, molecular-grade water, and 5 μL of extracted DNA template. The PCR was performed using a single hot start cycle at 94°C for 2 minutes, followed by 55 cycles of denaturation at 94°C for 15 s, annealing at 64°C for 15 s, and extension at 72°C for 15 s, followed by a single cycle at 72°C for 30 s.

RESULTS
Previously extracted DNA from the two individuals’ blood samples were sent to North Carolina State University, College of Veterinary Medicine, Intracellular Pathogens Research Laboratory (NCSC-CVM-IPRL). Anaplasma platys-like inclusion bodies had been previously visualized by a veterinary clinical pathologist working in Venezuela, who had experience with morulae identification in animal blood smears. Anaplasma platys DNA was PCR amplified from the two cases described previously. The PCR negative controls remained negative for all reactions. A 420 bp portion of the A. platys 16S rRNA gene was amplified from case 1 and sequence comparisons performed using the basic local alignment search tool (BLAST) against the GenBank database over a length of 350 nucleotides showed the highest sequence similarity (100% identical with 100% coverage) to A. platys 16S rRNA gene (AF286699). A 520 bp portion of the A. platys p44 gene was amplified from case 2 and sequence comparisons over a length of 450 bp had the highest sequence similarity (99% identical with 100% coverage) to A. platys p44 (GQ868750.1). The PCRs designed to amplify E. canis and A. phagocytophilum were negative.

DISCUSSION
Molecular diagnostic evidence supporting A. platys infection was recently reported by Maggi and others in a woman from Grenada, who had experienced migraines and progressively severe seizures, including status epilepticus. As the woman was co-infected with A. platys, Bartonella henselae, and Candidatus Mycoplasma haematoparvum, the extent to which each organism contributed to the patient’s symptoms could not be determined; however, after a 6-month course of doxycycline, A. platys, and Candidatus Mycoplasma haematoparvum DNA were no longer PCR amplified from the patient’s blood, whereas B. henselae DNA persisted, despite the long antibiotic course. In the current study, we report two additional cases of A. platys infection that were initially identified by visualizing intra-platelet inclusion bodies. Subsequently, stored, extracted DNA was used for PCR amplification and DNA sequencing to confirm A. platys infections, however, each with a different gene target. One possible explanation for this is the large amount of human genomic DNA (200–1,200 ng/μL) in the extractions, which can interfere with amplification of small amounts of pathogen DNA. Anaplasma platys p44 was amplified from the undiluted sample in case 2, which contained the least amount of human genomic DNA (200 ng/μL); this may have contributed to the success of the A. platys p44 PCR reaction for case 2. The A. platys p44 partial gene could not be amplified from case 1, despite diluting the highly concentrated DNA. Potentially, pathogen DNA was diluted beyond the detection of that PCR assay. Following dilutions, 16S rDNA PCR was performed on all samples. In addition to amplifying A. platys from case 1, a faint amplicon of the appropriate size was generated from case 2; however, a 16S rDNA sequence could not be obtained from case 2. Therefore, it is likely gene targets, 16S rDNA, and A. platys p44, were amplified from case 2, although sequence confirmation was obtained for only one amplicon. Further supporting an A. platys infection, both women had a history of peri-domestic tick exposure, which in nearly all instances is a result of R. sanguineus, the likely vector for A. platys in Venezuela, and throughout the world.

Anaplasma platys, (formerly Ehrlichia platys) was described in 1978 by Harvey and others in Florida, as basophilic inclusion bodies observed in platelets from a thrombocytopenic dog. In 1992, A. platys inclusions in dogs from Maracaibo, Venezuela were reported for the first time by Arraga. Subsequent studies, using techniques such as BCS analysis, serological assays (IFAT and ELISA), transmission electron microscopy, and PCR have shown A. platys is a prevalent tick-borne infection in dogs living in Venezuela. In dogs, A. platys induces a disease called infectious canine cyclic thrombocytopenia (ICCT), however, most infected dogs are not clinically ill. Based upon PCR testing in another study, 14 dogs from Grenada, located 600 miles north of Venezuela, were infected with A. platys. Other tick-transmitted alpha proteobacteria that are most often found in animals produce mild to severe disease in people, depending upon the individual’s immune status. For example, Ehrlichia chaffeensis and E. ewingii, transmitted by Amblyomma americanum ticks in North America, are more likely to cause severe and potentially life-threatening disease in immune compromised patients. Similarly, E. muris-like infection was recently recognized for the first time as a human pathogen in immune compromised patients, primarily transplantation recipients. Infection with E. canis, a cause of canine monocytic ehrlichiosis, has also been implicated as a cause of
mild disease in people from Venezuela.\textsuperscript{27} \textit{Ehrlichia canis} DNA was not amplified from either patient in this report.

For decades \textit{A. platys} was thought to only infect dogs; however, in 2005 \textit{A. platys}-like inclusion bodies were detected in 7\% of the platelets in a thrombocytopenic cat from Brazil.\textsuperscript{28} Subsequently, in 2010, molecular studies in Brazil documented \textit{A. platys} infection in another thrombocytopenic cat that also had platelet inclusion bodies on a stained blood smear.\textsuperscript{29} In 2008, \textit{A. platys} infection was confirmed in a goat from Cyprus by PCR followed by DNA sequence analysis.\textsuperscript{30} It was not until 2013, that the first DNA sequence-confirmed human case of \textit{A. platys} infection was reported.\textsuperscript{8}

\textit{Anaplasma platys} infections have been reported in dogs throughout the world, including the Americas (United States, Venezuela, Grenada, Chile, Argentina, Costa Rica), Europe (France, Spain, Germany, Italy, Greece, Croatia), Africa and the Middle-East (Israel), Asia (Taiwan, Japan, China, Thailand, Turkey, Malaysia), Australia, and Oceania.\textsuperscript{24,31–38} therefore exposure to this rickettsial organism is widely distributed.

The 16S rDNA sequences for two different \textit{A. platys} strains from Venezuela have been characterized:\textsuperscript{3} \textit{A. platys} Venezuela (GenBank accession no.: AF287153), obtained from a dog in Maracaibo\textsuperscript{21} and \textit{A. platys} Lara (GenBank accession no.: AF399917), from Lara state.\textsuperscript{9} Both of these strains are 99\% identical to the 16S rDNA sequence obtained from case 1. Strain variation in pathogenicity has been proposed because of reports of more severe disease manifestations, attributed to \textit{A. platys} infections in dogs in Europe.\textsuperscript{23,31} However, as co-infections with multiple tick-borne pathogens are being increasingly recognized in dogs throughout the world, it is possible that the more severe disease manifestations in European dogs were caused by infection with another undocumented pathogen.\textsuperscript{3,39,40} In particular, the role of \textit{Bartonella} spp. as co-infecting hemotrophic pathogens has only been recently described in dogs from Europe.

\textit{Anaplasma platys} has not been confirmed as a cause of illness in humans; however, this study provides additional molecular evidence supporting \textit{A. platys} infection in people, and physicians should recognize it as a potentially new zoonotic pathogen transmitted by ticks that typically infect dogs. Prospective studies are warranted to define the molecular prevalence of \textit{A. platys} and to define whether other pathogenic organisms contribute to the platelet-inclusions described in human patients in Venezuela.

Received June 16, 2014. Accepted for publication August 12, 2014. Published online September 29, 2014.

Note: During the submission, review and publication of this manuscript, the NCSU-CVM-IPRL, in collaboration with the University of Texas Medical Branch Galveston, published another manuscript describing infection with \textit{Anaplasma platys}, \textit{Ehrlichia chaffeensis} and \textit{Ehrlichia ewingii} in a dog, mother and daughter residing in North America. The reference citation for that manuscript is as follows: Breitschwerdt EB, Hegarty BC, BA, Qurollo BA, Saito TB, Maggi RG, Blanton LS, Bouyer BH. Intravascular persistence of \textit{Anaplasma platys}, \textit{Ehrlichia chaffeensis}, and \textit{Ehrlichia ewingii} DNA in the blood of a dog and two family members. Parasites and Vectors 2014;7:298–305.

Acknowledgments: We thank Tonya Lee for editorial assistance.

Financial support: Revenues of the VBDDL diagnostic service along with donations from industry and private sponsors are used for research projects otherwise unfunded that are pursued for their intellectual or societal benefits. Barbara Qurollo is a research postdoctoral fellow at North Carolina State University funded by IDEXX Laboratories Inc., Westbrook, ME.

Disclaimer: The authors have no conflicts of interest related to this work.

Authors’ addresses: Cruz M, Arraga-Alvarado, Omaira C. Parra, and Maribel A. Berreta, Unidad de Investigaciones Clinicas, Facultad de Ciencias Veterinarias, Universidad del Zulia, Maracaibo, Venezuela, E-mails: cruzmariaarragadelvarado@gmail.com, opamalado@gmail.com, and maribelberretuel@gmail.com. Barbara A. Qurollo, Barbara C. Hegarty, and Edward B. Breitschwerdt, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, E-mails: baqurollo@ncsu.edu, barbara_hegarty@ncsu.edu, and ed_breitschwerdt@ncsu.edu.

Reprint requests: Edward B. Breitschwerdt, College of Veterinary Medicine, North Carolina State University, 1060 William Moore Dr., Raleigh, NC 27607.

REFERENCES


