Intravascular persistence of *Anaplasma platys*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii* DNA in the blood of a dog and two family members

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Abstract

**Background:** Anaplasmosis, caused by *Anaplasma phagocytophilum* and *Anaplasma platys*, and ehrlichiosis, caused by *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, the “Panola Mountain *Ehrlichia*” and *Ehrlichia murs*-like pathogens have been identified as emerging tick borne infectious diseases in dogs and human patients. Persistent intravascular infection with these bacteria is well documented in dogs, but is less well documented in human beings.

**Methods:** Serology and PCR targeting multiple microbial genes, followed by DNA sequencing, was used to test sequential blood samples. Tissue culture isolation was attempted in two laboratories.

**Results:** *A. platys*, *E. chaffeensis*, and *E. ewingii* DNA was amplified from two *Anaplasma* and *Ehrlichia* seronegative family members and their dog, all lacking typical symptoms of anaplasmosis or ehrlichiosis. Following treatment with doxycycline, the dog and mother were *Anaplasma* and *Ehrlichia* spp. PCR negative.

**Conclusions:** Sequential PCR testing provided molecular evidence supporting intravascular persistence of *A. platys* and *Ehrlichia* spp. in two humans and their dog. Diagnosticians and clinicians should consider the potential for co-infections due to these tick borne organisms.

**Keywords:** *Anaplasma*, *Ehrlichia*, Rickettsiemia, PCR, DNA sequencing

**Background**

Anaplasmosis, caused by *Anaplasma phagocytophilum* and *A. platys*, and ehrlichiosis, caused by *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, *E. murs* and the “Panola Mountain *Ehrlichia*”, are emerging infectious diseases affecting dogs and human patients in North America and other parts of the world [1-3]. Canine vector borne diseases (CVBDs), including co-infections with more than one CVBD pathogen, are common in dogs, particularly those with frequent or constant vector exposure to varied tick species [4-6]. Among various animal species, including humans, *Anaplasma* and *Ehrlichia* spp. can induce acute, self-limiting or fatal infections, while persistent non-clinical infections often occur in dogs [7-9]. Following tick transmission, dogs can remain infected with *A. phagocytophilum*, *A. platys*, *E. canis*, *E. chaffeensis*, and *E. ewingii* for months to years, prior to immunological or therapeutic elimination of the infection or the development of chronic debilitating disease manifestations [7-9]. Dogs are natural hosts for *A. platys*, *E. canis* and potentially *E. ewingii*, but are considered opportunistic hosts for other *Anaplasma* and *Ehrlichia* spp. [6,7]. Persistent *E. chaffeensis* [10,11] and *E. canis* [12,13] human infections have been suspected previously. Recently, *A. platys* DNA was PCR amplified from blood samples collected from a veterinarian one month apart [14]. With the advent of PCR testing, reports of long-term *Anaplasma* and *Ehrlichia* human blood borne infections may become more common.

The data in this manuscript was generated after a physician requested to be entered into a *Bartonella* sp. research study. At time points spanning a six-month period, blood, buffy coat and inoculated cell cultures from members of the household, tested by PCR and
sequencing, identified combinations of *A. platys* and *Ehrlichia* species. Anaplasma platys, *E. chaffeensis*, and *E. ewingii* DNA was amplified and sequenced from the dog, the physician and her daughter’s blood, but not from two other household members.

**Methods**

**Patients and clinical presentation**

In September 2011, a 57-year-old-female physician requested to be entered into an IRB approved research study (North Carolina State University, 164-08-05), investigating the prevalence of *Bartonella* sp. bacteremia in various patient populations. In 2008, the woman had developed intermittent subcutaneous edema and mildly increased liver enzyme activities. Her 16-year-old daughter, adopted from China at 6 months of age, had been healthy until 2008, after which she developed upper body muscle pain requiring treatment by a physical therapist. Between 2008 and 2011, both mother and daughter were examined by several specialist physicians; neither had symptoms or hematological abnormalities (thrombocytopenia) consistent with anaplasmosis or ehrlichiosis; and both were HIV negative.

In 2008 the family purchased a 15-week-old male Papillon from a Chicago pet store. The puppy originated from a Missouri breeding facility, a region endemic for tick borne *E. chaffeensis* and *E. ewingii*. Due to an acute illness, the puppy was hospitalized hours after purchase; pneumonia was diagnosed radiographically and an extended hospitalization period was required to effectively treat the pneumonia. Subsequently, aggressive behavior was observed, resulting in occasional bites of the mother and daughter but not the other household members. In 2012, the dog was diagnosed with microvascular dysplasia, a congenital, developmental abnormality common in small breed dogs, that can contribute to hepatic dysfunction and behavioral abnormalities, potentially explaining the tendency for the dog to bite.

**Bartonella alpha proteobacteria (BAPGM) enrichment blood culture/PCR**

In accordance with a prior study [15], three sample sets were collected within a 7-day period to enhance detection of *Bartonella* spp. DNA using the BAPGM platform. Using blood aseptically collected on Monday, Wednesday and Friday (mother in September 2011, December 2011 and January 2012), (daughter in January and February 2012) and (dog in December 2011 and January 2012), *Bartonella* spp. serology and BAPGM (*Bartonella* alpha Proteobacteria growth medium) enrichment blood culture testing for *Bartonella* spp. was performed, according to previously published protocols [15,16].

**Buffy coat preparation**

After the initial PCR amplification of *E. chaffeensis* DNA from the mother’s blood in September 2011, aseptically obtained ethylenediaminetetraacetic acid (EDTA)-anti-coagulated whole blood was processed in two formats (whole blood and buffy coat cells) in an effort to increase *Ehrlichia* sp. PCR sensitivity. Blood samples (mother, January, February and March) and (daughter, father and grandmother, March only) were sent concurrently to the NCSU-IPRL and UTMB for parallel tissue culture isolation attempts.

**Anaplasma/ehrlichia conventional PCR assays**

Genomic DNA was extracted using either the QIAsymphony SP (Qiagen, Valencia, CA) or the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) as per manufacturer recommendations. Each DNA extraction process included several negative control non-infected blood samples. To avoid DNA contamination of samples, DNA extraction and PCR sample preparation were performed in a room separate from the PCR amplification and gel analysis rooms, with a unidirectional work flow. Previously described *Anaplasm* and *Ehrlichia* 16S rRNA gene conventional PCR (cPCR) assays were used to test whole blood, buffy coat and cell culture supernatant fractions prepared from the mother, father, daughter, grandmother and dog [17,18]. Amplifications were performed in a Mastercycler EPgradient® aluminum block thermocycler (Eppendorf, North America). GEPs and GEPr and GEPs and GEP1060 primers were used respectively, to amplify 420 and 973bp segments of the 16S rRNA gene [17] using *Ehrlichia canis* DNA as a positive control. Subsequently, newly introduced or derived (*sodB*) PCR assays were used by a blinded IPRL researcher to target the *A. platys* *p44* gene, *E. chaffeensis* and *E. ewingii* p28 genes, and *Ehrlichia* spp. *sodB* gene in blood and cell culture supernatants. Primers and cPCR conditions are provided in Table 1. Amplicons were assessed by electrophoresis of 8 μl of each product in 2% agarose gels containing ethidium bromide. DNA extraction and PCR negative controls remained negative throughout the study.

**DNA sequencing**

Amplicons were sequenced directly or cloned into plasmid pGEM-T Easy Vector System Promega® (Madison, WI) by GENEWIZ Inc. (Research Triangle Park, NC). Sequences were aligned and compared with GenBank sequences using AlignX software (Vector NTI Suite 6.0, InforMax, Inc.).

**Serologic testing**

After amplification of *E. chaffeensis* DNA from the mother’s blood, her physician requested *E. chaffeensis* serology from a commercial laboratory (Quest Diagnostics,
Table 1 Conventional PCR primer sequences and thermocycler conditions for assays used in this study

| Target gene       | Size in base pairs (bp) | Primers and names and sequence, reaction mix and run conditions                                                                 |
|-------------------|-------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| Ehrlichia or Anaplasma 16S rRNA | 420 bp                  | **GEPS** 5'-CTG GCG GCA AGC YTA ACA CAT GCA AGT CGA ACG GA 3' **GEPr** 5'-CTT CTT TRG GTA TTA TCT TCC CTA YYG 3'  For 25 μl mix: 12.5 μl MyTaq HS Mix (Bioline), 0.2 μl 100 μM each primer (IDT® DNA Technology), 7.3 μl molecular grade water, + 5 μl template DNA. Denaturation 2 min @95°C, then 55 cycles 15 sec @94°C, annealing 10 sec @64°C, extension 15 sec @72°C, final extension 30 sec @72°C. |
| Ehrlichia or Anaplasma 16S rRNA | 973 bp                  | **GEPS** 5'-CTG GCG GCA AGC YTA ACA CAT GCA AGT CGA ACG GA 3' **LongGEP1060r** 5'-CTG TGT RAG GTC CAG CCG AAC TGM SYC 3' As above except annealing and extension times extended to 25 and 30 sec. |
| Ehrlichia spp. sodb | 300 bp                  | **sodbF** 5'-TTT AAT AAT GCT GGT CAA GTA TGG AAT CAT **sodbR** 5'-AAG GGT AAT ACA T |
| E. chaffeensis p28 | 590 bp                  | **EchP28F** 5'-GAC CCA ACA AGT GGT ATT AAC GG **EchP28R** 5'-CTG GCC TAG AGT AAC CTA AC  For 25 μl mix: 12.5 μl MyTaq HS Mix (Bioline), 0.25 μl 50 μM each primer (Sigma-Aldrich), 7 μl molecular grade water + 5 μl template DNA. Single hot start cycle 3 min @95°C, then 55 cycles denaturation 10 sec @94°C, annealing 15 sec @58°C, extension 15 sec @72°C, then a single cycle 30 sec @72°C. |
| E. ewingii p28 | 215 bp                  | **EEM2F** (Ref 32) 5'-GGA GCT AAA ATA GAA GAT AAT C **EEM1R** 5'-GGT CCA AAA GGT AAT ACA T  For 25 μl mix: 12.5 μl of MyTaq HS Mix (2X) (Bioline), 0.25 μl 50 μM each primer (Sigma-Aldrich), 2 μl molecular grade water + 10 μl template DNA. Single hot start cycle 3 min @95°C, then 55 cycles denaturation at 15 sec @94°C, annealing 15 sec @56°C, extension 30 sec @72°C, then a single cycle 1 min @72°C. |
| A. platys p44 | 520 bp                  | **Apl_p44F3** 5'-GCT AAG TGG AGC GGT GGC GAT GA CAG **Apl_p44R3** 5'-CGA TCT CCG CTT TCG TAT TCT TC  For 25 μl mix: 12.5 μl MyTaq HS Mix (2X) (Bioline), 0.3 μl 50 μM each primer (Sigma-Aldrich), 2 μl molecular grade water + 10 μl template DNA. Single hot start cycle 3 min @95°C, then 55 cycles denaturation 15 sec @94°C, annealing 10 sec @70°C, and extension 30 sec @72°C, then a single cycle 1 min @72°C. |

Nichols Institute, Valencia, CA); otherwise human serological testing was performed at the Rickettsial Disease Laboratory, UTMB. Serum specimens from all family members from multiple collection dates were serially diluted (twofold) from 1:64 to 1:2048 and tested for *E. chaffeensis* IgG class antibodies by ImmunoFluorescent assay (IFA) with Alexa Fluor 488 goat-anti-human second-ary antibody (dilution 1:1000). An IFA titer of 1:64 or higher was considered positive. Serum from the dog was tested for *Ehrlichia* antibodies by IFA in the IPRL, using antigens derived from canine monocytic DH82 cultures infected with *E. canis* and *E. chaffeensis* and for *Anaplasma* or *Ehrlichia* antibodies using the Snap® 4Dx®, (IDEXX Laboratories Inc., Westbrook, Maine) [19,20].

Cell culture isolation
Parallel isolation attempts were made using white blood cell pellets (WBC) into various cell lines at NCSU-IPRL and UTMB. EDTA anti-coagulated whole blood from each individual was lysed with either ACK lysis buffer or hypotonic saline and centrifuged at UTMB or centrifuged in two stages at IPRL to prepare WBC pellets which were suspended in medium and dispersed into 25 cm² flasks containing DH82 and 1ES6 tick cells (IPRL) or DH82, RF/6A, Vero, HEL299 and C6/36 cells (UTMB) and incubated at 37°C in 5% CO₂. Negative control flasks were maintained in parallel. Cytology (Diff Quik or Gimenez staining) and PCR were sequentially performed.
Results

Exposure history
The family resided in suburban Chicago. There were no forested areas that might contain tick-infested deer for at least 1 mile in any direction. The mother did not recall any tick infestations in the home before or after purchasing the puppy and no family member reported a history of tick attachment. Occasional raccoon, skunk, opossum, coyote and other small mammals and birds were seen in the neighborhood. The mother and daughter were the primary caregivers for the dog and the only family members to have experienced consistent dog contact and occasional bites.

Bartonella serology and enrichment culture/PCR
Based upon sequential testing, Bartonella spp. antibodies were not detected in the mother, daughter or dog’s serum specimens, and at all testing time points, BAPGM enrichment blood culture platform specimens (4 PCRs/sample/date of collection) were Bartonella sp. PCR negative. Thus, there was no serological or PCR evidence to support Bartonella spp. exposure or infection.

Anaplasma/Ehrlichia conventional 16S rRNA PCR and sequencing
Using 16S GEP primers that amplify both Anaplasma and Ehrlichia species, Ehrlichia chaffeensis 16S rDNA was cPCR amplified and sequenced from one of three September 2011 blood specimens (Table 2). The 16S rDNA sequence shared 99.7% (359/360 base pair) similarity with E. chaffeensis strain Arkansas CP000236 (Table 3). Based upon this E. chaffeensis result, additional blood samples from the mother, dog, daughter, father and maternal grandmother were submitted for testing in the IPRL on five, four, three, two and one occasion, respectively between September 2011 and March 2012.

Anaplasma/ehrlichia p28, p44 and sodb gene amplification and DNA sequencing
To confirm the 16S rDNA PCR results, other Anaplasma and Ehrlichia spp. genes were targeted, using freshly extracted, stored, whole blood and WBC preparations. The initial A. platys, E. chaffeensis and E. ewingii DNA amplification results were confirmed by cPCR amplification and DNA sequencing of the p44, p28, and sodb genes (Table 3).

Serology
When tested by a commercial laboratory in February 2012, the mother was reportedly E. chaffeensis seroreactive (IgM < 1:40, IgG 1: 160); however, at UTMB, five sera collected between September 2011 and March 2012 (mother) and February and March (daughter) were not E. chaffeensis seroreactive at a 1:64 dilution by IFA testing. Western immunoblotting also did not identify Ehrlichia spp. antibodies. When tested by the IPRL in January 2012, the dog was not E. canis, Babesia canis, Babesia gibsoni, Bartonella henselae, Bartonella vinsonii subsp. berkholzii, Leishmania infantum, or Rickettsia rickettsii IFA seroreactive and was also ELISA negative for Dirofilaria immitis.

Table 2 Summary of conventional PCR amplification and DNA sequencing results from whole blood, serum, WBC fractions and DH82, RF/6A or IES6 cell cultures tested between September 2011 and June 2012

| Date   | Mother | Dog  | Daughter | Father/Grandmother |
|--------|--------|------|----------|-------------------|
|        | Ech    | Eew  | Apl      | Ec                |        |
| 09/2011| +#     | -    | -        | NT                | NT     |
| 12/2011| +#     | +*   | +#*      | +#                | NT     |
| 01/2012| +#     | +#*  | +#*      | -                 | NT     |
| 02/2012| +*     | -    | -        | +#                | NT     |
| 03/2012| +*     | -    | -        | -                 | NT     |
| 06/2012| -      | -    | -        | -                 | NT     |

Sample source or sources are designated for each testing time point.
Ech = Ehrlichia chaffeensis, Eew = Ehrlichia ewingii, Apl = Anaplasma platys, Ec = Ehrlichia canis.
All + results confirmed by DNA sequencing.
# = amplicon obtained from blood, serum or buffy coat fraction.
* = amplicon obtained from DH82, RF/6A or IES6 cell cultures.
NT = not tested.
** = post doxycycline treatment.
NA = not an applicable gene target for the particular Ehrlichia species.

Venezuela dog strain, sequences: A. platys 16S validated by other NR_044747 from an Oklahoma dog strain, were not visualized. The daughter was not treated. When retested in June 2012, blood and tissue culture with doxycycline 5 mg/kg every 12 hours for 5 weeks. 100 mg PO BID for 15 days and the dog was treated. In May 2012, the mother was treated with doxycycline treatment during 8 successful isolation or evidence of organism amplification intracellular bacteria or morulae, but did not result in generated Diff Quik or Gimenez staining indications of Organism isolation efforts at both NCSU and UTMB = E. chaffeensis. Based upon DNA sequence similarities, specimens from the dog and both family members contained the same genotypes.

Ech = Ehrlichia chaffensis. Eew = E. ewingii. Apl = Anaplasma platys. NA = not an applicable gene target for the particular Anaplasma or Ehrlichia species.

Numbers represent % sequence identity to the following GenBank reference Eech Eew Apl Ech Eew Apl Ech Eew Apl

| Gene target | Mother | Daughter | Dog |
|-------------|--------|---------|-----|
| GEP 16S     | 99.7   | 99.7    | 99.9 |
| sodB        | 100    | 99.2    | NA  |
| P28         | 96.6   | 100     | NA  |
| P44         | NA     | NA      | 99.6 |

Based upon DNA sequence similarities, specimens from the dog and both family members contained the same genotypes.

Numbers represent % sequence identity to the following GenBank reference Eew Ech Eew Apl Eew Eew Apl Eew

Discussion and conclusions

This study provided PCR amplification and sequencing evidence supporting the persistence of A. platys, E. chaffeensis and E. ewingii DNA in the blood of a dog and two family members. Specifically, during the six month study period, E. chaffeensis, E. ewingii, and A. platys DNA was amplified and sequenced from the mother's blood and/or cell culture supernatant specimens at 5, 3, and 2 time points, respectively. Anaplasma and Ehrlichia gene targets not routinely assayed in our laboratory confirmed the initial identification of pathogen DNA in all three family members. Furthermore, the partial gene sequences amplified between family members were identical, suggesting they were infected with the same pathogen genotypes. Despite repeated molecular documentation of these bacteria, Ehrlichia spp. IFA antibody titers and Western immunoblotting were negative at UTMB and the dog was not Anaplasma or Ehrlichia seroreactive at NCSU-IPRL. As A. platys and E. ewingii have not been successfully cultivated in cell culture, IFA serological assays were not available for testing purposes. Whether Anaplasma and Ehrlichia spp. co-infection altered the expected humoral immune response, whether assay antigens were not well matched with the infecting genotypes or whether anergy played a role in seronegativity remains unknown. Similar comparative correlations between canine and human vector-borne infections can be found in the literature. Persistent infections, spanning months to years, with A. platys, E. chaffeensis and E. ewingii, have been frequently reported in dogs [6,7,18,21]. In contrast, there is limited evidence supporting persistent human E. chaffeensis infections [10,11]. Based upon challenge studies, dogs can be experimentally re-infected with a homologous or heterologous E. canis isolate, thus infection does not infer protective immunity in dogs [22]. Re-infection with E. chaffeensis has also been reported in a liver transplant patient [23]. In conjunction with improved diagnostic testing modalities, co-infections of vector-borne diseases have been reported in dogs and in human patients. As dogs are more frequently exposed to ticks than their human counterparts, co-infections are more often reported in dogs [5-7,19] however, among other examples, E. chaffeensis and Rickettsia rickettsii co-infection was reported in a 44-year-old man [24]. Experimentally, A. platys and E. canis co-infections in dogs [18], influenced the patterns and severity of hematologic and serologic findings.

To confirm and validate the initial, unexpected 16S rDNA PCR results from the mother and subsequently the daughter and dog, other gene targets were amplified and sequenced, isolation was attempted in two laboratories, and two investigators tested samples independently and at different time points. Because microbial-specific genes were targeted, the same PCR assays are applicable for testing human and veterinary patient populations and vectors.
for the presence of bacterial DNA. PCR has limitations. False negative results occur when testing samples with low template concentrations and selective amplification of the predominant organism can occur in patients co-infected with genetically similar organisms. By comparing PCR results from different sample sources (blood, serum, Buffy coat, and tissue culture extracts), the assays used in this study may not have been sensitive enough to consistently detect these bacteria within blood specimens collected during the same week (data not shown), illustrating the need to enhance the sensitivity of Anaplasma and Ehrlichia PCR, particularly when patient samples contain DNA sequences of genetically related organisms. DNA carryover or amplicon contamination within a laboratory may result in false positives [25,26]. Importantly, throughout this study, all DNA extraction and PCR-negative controls remained negative. Furthermore, PCR testing of blood, serum, WBC, and tissue culture extracts from the father or maternal grandmother did not identify Anaplasma and Ehrlichia sp. DNA, and both the dog and mother became PCR negative after treatment with doxycycline, a finding that supports infection with viable organisms.

Nearly two decades ago investigators in Venezuela described inclusions in human platelets, a subset of which were ultrastructurally consistent with A. platys [27,28]. Until recently [14], no subsequent report of human A. platys infection was published in the English literature. There is substantial epidemiological support for Rhipicephalus sanguineus (the brown dog or kennel tick) as the vector and the dog as the primary reservoir host for A. platys and E. canis [20]. As the puppy in this study originated from a kennel in Missouri, exposure to R. sanguineus seemed more likely than exposure to A. americanum, which is a field tick that will feed on numerous animal species [29]. Although vector competence has not been proven, E. chaffeensis DNA was amplified from 56% of R. sanguineus obtained from dogs and puppies housed in a kennel in Cameroon, a country where both canine and human infection with E. chaffeensis has been previously reported [30]. In contrast, recent efforts to experimentally transmit E. ewingii by R. sanguineus were not successful [31]. The source of A. platys, E. chaffeensis and E. ewingii infection in the pet and the family members and E. canis in the dog remains unknown; however, this puppy originated from a kennel in a highly endemic state for tick transmission of E. chaffeensis and E. ewingii by A. americanum [1,29] and A. platys and E. canis are transmitted by a tick (R. sanguineus) that infests dogs in kennels.

In summary, A. platys and Ehrlichia spp. DNA was documented in an unusual familial cluster involving a dog and two family members. Future studies should determine whether repeated documentation of DNA of these organisms reflects ongoing infection and whether there are medical consequences associated with the persistence of DNA of these organisms. To guide testing and treatment decisions, sequential PCR testing of blood, WBC or cell culture-enhanced samples may improve DNA-detection sensitivity.

Abbreviations
BAPGM: Bartonella alpha proteobacteria growth medium;
EDTA: Ethylenediaminetetraacetic acid.

Competing interests
In conjunction with Dr. Sushama Sontakke and North Carolina State University, Dr. Breitschwerdt holds U.S. Patent No. 7,115,385; Media and Methods for cultivation of microorganisms, which was issued October 3, 2006. He is the chief scientific officer for Galaxy Diagnostics, a company that provides advanced diagnostic testing for the detection of Bartonella species infection in animals and humans. All other authors have no potential conflict. In the context of this manuscript, none of the authors disclose any conflicts of interest.

Authors’ contribution
EBB coordinated communications and generated the initial draft manuscript. BCH, BAQ, and RGM performed tissue culture isolation and PCR testing at North Carolina State University. TBS, LSB, and DHB performed tissue culture isolation and serological testing at the University of Texas Medical Branch at Galveston. All authors contributed to the content of the manuscript and all authors reviewed the final submission.

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References
1. Demma LJ, Holman RC, McQuiston JH, Krebs JW, Swerdlow DL: Epidemiology of human ehrlichiosis and anaplasmosis in the United States, 2001–2002. Am J Trop Med Hyg 2005, 73:400–409.
2. Nicholson WL, Allen KE, McQuiston JH, Breitschwerdt EB, Little SE. The increasing recognition of rickettsial pathogens in dogs and people. Trends Parasitol 2010, 26(4):205–212.
3. Pitt B, Sloo LM, Johnson DK, Munderloh UG, Paskevitz SM, McElroy KM, McFadden JD, Brinner MJ, Netzel DF, Liu G, Nicholson WL, Nelson CM, Franson JJ, Martin SA, Cunningham SA, Steward CR, Bogumill K, Bjorgaard ME, Davis JP, McQuiston JH, Warshauer DNA, Wilhelm MP, Patel R, Trivedi VA, Eremeeva ME. Emergence of a new pathogenic Ehrlichia species, Wisconsin and Minnesota, 2009. N Engl J Med 2011, 365:422–429.
4. de Caprariis D, Dantas-Torres F, Capelli G, Memecke N, Staneck D, Breitschwerdt EB, Otranto D: Evolution of clinical, haematological and biochemical findings in young dogs naturally infected by vector-borne pathogens. Vet Microb 2011, 149:206–212.
5. De Tommasi AS, Otranto D, Dantas-Torres F, Capelli G, Breitschwerdt EB, de Caparrias O. Are vector-borne pathogen co-infections complicating the clinical presentation in dogs? Parasit Vectors 2013, 6:97.

6. Korrick SK, Breitschwerdt EB, Hegarty BC, Southwick KL, Colitz CM, Hancock SL, Bradley JM, Rumbough R, McPherson JT, MacCormack JN. Coinfection with multiple tick-borne pathogens in a Walker Hound kennel in North Carolina. J Clin Microbiol 1999, 37:2631–2638.

7. Breitschwerdt EB, Hegarty BC, Hancock SL. Sequential evaluation of dogs naturally infected with Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia equi, Ehrlichia ewingii, or Bartonella vinsonii. J Clin Microbiol 1998, 36:2645–2651.

8. Hegarty BC, Maggi RG, Koskinen P, Beall MJ, Eberts M, Chandrashekar R, Breitschwerdt EB. Ehrlichia muus infection in a dog from Minnesota. J Vet Intern Med 2012, 26:1217–1220.

9. Scorpio DG, Dumler JS, Barat NC, Cook JA, Barat CE, Stillman BA, DeBiscigo KC, Beall MJ, Chandrashekar R. Comparative strain analysis of Anaplasmaphagocytophilum infection and clinical outcomes in a canine model of granulocytic anaplasmosis. Vector Borne Zoonotic Dis. 2011, 11:223–229.

10. Dumler JS, Saltik WL, Walker DH. Persistent infection with Ehrlichia chaffeensis. Clin Infect Dis. 1993, 17:903–905.

11. Roland WE, McDonald G, Caldwell CW, Everett ED. Ehrlichiosis: a cause of prolonged fever. Clin Infect Dis 1995, 20:821–825.

12. Perez M, Rikihisa Y, Wen B. Ehrlichia canis-like agent isolated from a man in Venezuela: antigenic and genetic characterization. J Clin Microbiol 1996, 34:2133–2139.

13. Unver A, Perez M, Orellana N, Huang H, Rikihisa Y. Molecular and antigenic comparison of Ehrlichia canis isolates from dogs, ticks, and a human in Venezuela. J Clin Microbiol 2001, 39:2788–2793.

14. Maggi RG, Mascarelli PE, Havenga LN, Naidoo V, Breitschwerdt EB. Co-infection with Anaplasmaphagocytophilum, Bartonella henselae and Candidatus Mycoplasma haematoparum in a veterinarian. Parasit Vectors 2013, 6:103.

15. Pultorak EI, Maggi RG, Mascarelli PE, Breitschwerdt EB. Serial testing from a 3-day collection period by use of the Bartonella Alphaproteobacteria Growth Medium Platform may enhance the sensitivity of Bartonella species detection in bacteremic human patients. J Clin Microbiol 2013, 51(6):1673–1677.

16. Maggi RG, Mascarelli PE, Pultorak EL, Hegarty BC, Bradley JM, Mozayeni BR, Breitschwerdt EB. Bartonellasp. bacteremia in high-risk immunocompetent patients. Diagn Microbiol Infect Dis 2011, 71:450–457.

17. Eddlestone SM, Diniz PVP, Neer TM, Gaunt SD, Converse R, Cho D, Hosgood G, Hegarty B, Breitschwerdt EB. Doxycycline clearance of experimentally induced chronic Ehrlichia canis infection in dogs. J Vet Intern Med 2007, 21:1237–1242.

18. Gaunt S, Beall M, Stillman B, Lorentzen L, Diniz P, Chandrashekar R, Breitschwerdt EB. Experimental infection and co-infection of dogs with Anaplasmaphagocytophilum and Ehrlichia canis: hematologic, serologic and molecular findings. Parasit Vectors 2010, 3:33.

19. Chandrashekar R, Mainville CA, Beall MJ, O’Connor T, Eberts MD, Alleman AR, Gaunt SD, Breitschwerdt EB. Performance of a commercially available in-clinic ELISA for the detection of antibodies against Anaplasmaphagocytophilum, Ehrlichia canis, and Borellia burgdorferi and Dirofilaria immitis antigens in dogs. Am J Vet Res 2010, 71:1443–1450.

20. Dindz PP, Beall MJ, Omark K, Chandrashekar R, Daniluk DA, Gyr KE, Koteniski JF, Robbins RG, Lalo PG, Hegarty BC, Breitschwerdt EB. High prevalence of tick-borne pathogens in dogs from an Indian reservation in northeastern Arizona. Vector Borne Zoonotic Dis 2010, 10:117–123.

21. Eddlestone SM, Gaunt SD, Neer TM, Boudreaux CM, Gill A, Haschke E, Converse RE. PCR detection of Anaplasmaphagocytophilum in blood and tissue of dogs during acute phase of experimental infection. Exp Parasitol 2007, 115:205–210.

22. Breitschwerdt EB, Hegarty BC, Hancock SI. Doxycycline hyclate treatment of experimental canine ehrlichiosis followed by challenge inoculation with two Ehrlichia canis strains. Antimicrob Agents Chemother 1998, 42:362–368.

23. Liddell AM, Sumner JW, Paddock CD, Rikihisa Y, Unver A, Buller RS, Storch GA. Reinfection with Ehrlichia chaffeensis in a liver transplant recipient. Clin Infect Dis 2002, 34:1644–1647.

24. Sexton DJ, Corey GR, Carpenter C, Kong LQ, Ghandi T, Breitschwerdt EB, Hegarty B, Chen SM, Feng HM, Yu XJ, Olano J, Walker DH, Dumler SJ. Dual infection with Ehrlichia chaffeensis and a spotted fever group rickettsia: a case report. Emerg Infect Dis 1998, 4:311–316.

25. Dong J, Olano JP, McBride JW, Walker DH. Emerging pathogens: challenges and successes of molecular diagnostics. J Mol Diagn 2008, 10:185–197.

26. Sontakke S, Cadenas MB, Maggi RG, Diniz PP, Breitschwerdt EB. Use of broad range16S rDNA PCR in clinical microbiology. J Microbiol Methods 2009, 76:217–225.

27. Arraga-Alvarado C, Montero-Ojeda M, Bernardoni A, Anderson BE, Parra O. Human ehrlichiosis: report of the 1st case in Venezuela. Invest Clin 1996, 37:35–49.

28. Arraga-Alvarado C, Palmar M, Parra O, Salas P. Fine structural characterization of a Rickettsia-like organism in human platelets from patients with symptoms of ehrlichiosis. J Med Microbiol 1999, 48:991–997.

29. Childs JE, Paddock CD. The ascendency of Amblyomma americanum as a vector of pathogens affecting humans in the United States. Annu Rev Entomol 2003, 48:307–337.

30. Ndip LM, Ndip RN, Esermu SN, Walker DH, McBride JW. Predominance of Ehrlichia chaffeensis in Rhipicephalus sanguineus ticks from kennel-confined dogs in Limbe, Cameroon. Exp Appl Acarol 2010, 50:165–168.

31. Yabsley MJ, Adams DS, O’Connor TP, Chandrashekar R, Little SE. Experimental primary and secondary infections of domestic dogs with Ehrlichia ewingii. Vet Microbiol 2011, 150:315–321.

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