In the United States, tickborne Rickettsia parkeri, R. philipii (Rickettsia 364D), and R. rickettsii, causative agents of Rocky Mountain spotted fever (RMSF), are well-documented human spotted fever group (SFG) rickettsioses (1). R. rickettsii is the only known cause of SFG rickettsioses in dogs (2). The extent to which other SFG Rickettsia are pathogenic in dogs is unclear; however, SFG Rickettsia seroprevalence is high among dogs in the United States and Mexico (3,4). The increased R. rickettsii seroprevalence in humans in the United States during the past decade has been attributed to SFG Rickettsia cross-reactivity (1,5).

We report 3 dogs with febrile illness located in different US states. Samples from the dogs were R. rickettsii seroreactive. Identical Rickettsia DNA gene sequences were obtained from each dog’s blood specimen and used to investigate Rickettsia spp.

The Cases
On May 15, 2018, a 10-year-old male neutered mixed breed dog (case 1) from Tennessee was examined by a veterinarian for lethargy and hyporexia. The owner reported removing a tick (species unknown) within the previous 2 weeks. On physical examination, the dog had fever (39.8°C) and possible hepatomegaly. Radiographic imaging results were unremarkable. Thrombocytopenia was the only abnormality noted on complete blood count (CBC). Serum biochemistry panel (SBP) abnormalities included hyperglobulinemia, increased serum alkaline phosphatase activity, hypoglycemia, and hyponatremia (Table 1). Results of urine dipstick and sediment examination were unremarkable. The dog’s samples were R. rickettsii seroreactive and PCR positive for Rickettsia (Table 2). Clinical abnormalities resolved after treatment with doxycycline, and the dog remained healthy during the 1-year follow-up period.

On May 8, 2019, a 9-year-old male neutered Boston terrier (case 2) from Illinois was examined by a veterinarian for lethargy, difficulty walking, and painful elbows. Clinical signs developed 3 days after returning from a tick-infested area in Arkansas. Abnormalities noted on physical examination included fever (40.1°C), dehydration, joint effusion, elbow pain, and shifting leg lameness. Thrombocytopenia and mild leukocytosis were the only CBC abnormalities (Table 1). SBP abnormalities included hypoalbuminemia, increased alanine amino transferase activity, alkaline phosphatase activity, hypercholesterolemia, and hypocalcemia (Table 1). Mild microalbuminuria was noted. Neutrophilic inflammation was documented by synovial fluid cytology in the right and left stifle joints, right tarsus, and left elbow joint. The left carpus contained moderate, chronic inflammation with very rare extracellular cocci; however, culture resulted in no bacterial growth. The dog experienced cardiorespiratory arrest during sedated arthrocentesis but recovered after CPR and sedative reversal. Thoracic radiographs were unremarkable. The dog’s samples were R. rickettsii seroreactive and PCR-positive for Rickettsia and convalescent titers demonstrated 4-fold seroconversion (Table 2). Most clinical abnormalities resolved after administration of doxycycline to treat rickettsiosis, prednisone to treat potential immune-mediated component, omeprazole to prevent gastric ulcers, and metronidazole to treat...
assumed dysbiosis. All SBP changes resolved within 5 months of treatment and the dog remained healthy during the 5-month follow-up.

On August 28, 2019, a 9-year-old male neutered terrier mixed-breed (case 3) from Oklahoma was examined by a veterinarian for lethargy, hyporexia, and polydipsia. Physical examination revealed fever (39.8°C) and palpable abdominal tenderness. CBC abnormalities included a normocytic normochromic anemia and thrombocytopenia. SBP abnormalities included hypoproteinemia, hypocalcemia, and mild azotemia. A protein-losing nephropathy (PLN) was documented by urine dipstick and protein/creatinine ratio. Blood samples were R. rickettsii seroreactive and Rickettsia PCR positive, and convalescent titers demonstrated 4-fold seroconversion (Table 2).

### Table 1. Findings from physical examination, laboratory results, treatment regimens for 3 dogs infected with a novel *Rickettsia* species, United States*

| Examination and treatment | Case 1 | Case 2 | Case 3 |
|---------------------------|--------|--------|--------|
| Physical examination      | Febrile (39.8°C); lethargy; +/− hepatomegaly | Febrile (40.1°C); lethargy; dehydratation; joint effusion (elbow, carpus, and tarsus); arthropathy; shifting leg lameness | Febrile (39.8°C); lethargy; abdominal pain |
| CBC                       | Platelets 141 × 10^9 cells/µL (RI 200–500 × 10^9 cells/µL) | Platelets 139 × 10^9 cells/µL (RI 170–400 × 10^9 cells/µL) | Platelets 60 × 10^9 cells/µL (RI 125–500 × 10^9 cells/µL); Hct 35.2% (RI 36%–55%); 2 d later platelets 25 × 10^9 cells/µL and Hct 26.8% |
| SBP                       | Globulins 4.5 g/dL (RI 2.1–4.4 g/dL); ALP 177 U/L (RI 11–140 U/L); glucose 73 mg/dL (RI 75–125 mg/dL); sodium 136.5 mmol/L (RI 143–153 mmol/L) | Albumin 2.2 g/dL (RI 2.7–4.4 g/dL); ALT 1.158 U/L (RI 12–118 U/L); ALP 1.702 U/L (RI 5–131 U/L); cholesterol 352 mg/dL (RI 92–324 mg/dL); calcium 8.4 mg/dL (RI 8.9–11.4 mg/dL) | Albumin 1.0 mg/dL (RI 2.5–4.3 mg/dL); calcium 8.4 mg/dL (RI 8.9–11.4 mg/dL); BUN 35 (35, RI 7–28 mg/dL) |
| Urinalysis                | USG 1.007 | Microalbuminuria 3.1 (RI <2.5 mg/dL) | USG 1.033; 3+ proteinuria; UPC 14.7 (RI 0.00–1.00) |
| Treatment regimen         | Doxycycline (6 mg/kg every 12 h for 21 d) | Doxycycline (7 mg/kg every 12h for 28 d); prednisone (1 mg/kg every 12 h for 9 mo with gradual taper); omeprazole (1.4 mg/kg every 12h for 9 mo); ondansetron (0.5 mg/kg every 12h for 15 d); and metronidazole (17 mg/kg every 12 h for 15 d) | Doxycycline (7.5 mg/kg q12h for 40 d); prednisone (1 mg/kg every 12 h for 14 d, then 0.5mg/kg every 12 h for 6 d, then every 24 h for 22 d until death), mycophenolate (12.5 mg/kg every 12 h for 22 d until death) |

*ALP, alkaline phosphatase activity; ALT, alanine amino transferase activity; BUN, blood urea nitrogen; CBC, complete blood count; Hct, hematocrit; RI, reference interval; SBP, serum biochemistry panel; UA, urinalysis; UPC, urine protein/creatinine ratio; USG, urine specific gravity.

### Table 2. CVBD diagnostic results for blood and serum samples from 3 dogs infected with a novel *Rickettsia* species*

| Sample dates | CVBD panel† | IFA‡ | 23S-5S ITS | htrA (17kDa) | mmpA-purC ITS | gltA region | ompA region |
|--------------|-------------|------|------------|--------------|---------------|-------------|-------------|
| Case 1       |             |      |            |              |               |             |             |
| 2018 May 5†† | –           | 1.512| +          | +            | +             | +           | +           | –**         |
| Case 2       |             |      |            |              |               |             |             |
| 2019 May 8†† | –           | 1.256| +          | +            | +             | NA††        | +           | +           | NA††        |
| 2019 May 15  | –           | 1.819| NA         | NA           | NA            | NA          | NA          | NA          |
| 2019 May 28  | –           | 1.1024| NA         | NA           | NA            | NA          | NA          | NA          |
| 2019 Jul 16  | NA          | 1.2048| NA         | NA           | NA            | NA          | NA          | NA          |
| 2019 Oct 2   | –           | 1.2048| NA         | NA           | NA            | NA          | NA          | NA          |
| 2019 Nov 12  | –           | 1.2048| NA         | NA           | NA            | NA          | NA          | NA          |
| Case 3       |             |      |            |              |               |             |             |
| 2019 Aug 28††| –           | 1.0124| +          | +            | +             | +           | +           | +           |
| 2019 Sep 10  | NA          | 1.1892| NA         | NA           | NA            | NA          | NA          | NA          |

†CVBD, canine vectorborne disease; IFA, immunofluorescence assay; NA, not applicable; +, positive; −, negative.
‡Panel includes IFA serology for Babesia canis vogeli, B. gibsoni, Bartonella henselae, B. koehlerae, B. vinsonii berkholfi, and Ehrlichia canis; point-of-care ELISA serology test SNAP 4DX Plus for *Dirofilaria immitis* antigen and antibodies against *Anaplasma phagocytophilum*, *A. platys*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *E. ewingii*, and PCR for *Anaplasma*, *Babesia*, *Bartonella*, *Ehrlichia*, hemotropic *Mycoplasma*, *Neoehrlichia*, and *Neorickettsia*.

††IFA results are reported as reciprocal titers. All samples were positive for *R. rickettsii*.
§PCR assay gene targets 23S-5S ITS, htrA (17 kDa), mmpA-purC ITS, gltA, and ompA.
∥Sample tested before doxycycline treatment administered.
**The PCR was negative despite repeated attempts. ompA region 3 PCR assay was designed to bridge ompA regions 1 and 2 to obtain an additional 281 bps. The total amplon size of ompA region 3 is 533 bp (Appendix Table, https://wwwnc.cdc.gov/EID/article/26/12/20-0272-App1.pdf). DNA from case 1 was ≥1 year old when retrospective PCRs were performed. Poor DNA quality might have prevented amplification of the larger amplon.
†††PCR assays were not performed due to depleted blood sample for DNA extraction and testing.
‡‡‡GenBank accession nos. for sequences from case 3: 23S-5S ITS, MT050446; htrA (17 kDa), MT050446; mmpA-purC ITS, MT066187; gltA, MT050445; and ompA, MT050447.
The year before, in August 2018, the dog described in case 3 was examined by a veterinarian for lethargy after tick attachment. At that time, fever (39.7°C), anemia, thrombocytopenia, hyperbilirubinemia, and hypoproteinemia were documented. IFA serology tests performed by 1 diagnostic laboratory showed samples were R. rickettsii seroreactive (1:320) but seronegative for Anaplasma spp., Borrelia burgdorferi, and Ehrlichia spp. by SNAP 4Dx Plus (IDEXX Laboratories, https://www.idexx.com). Doxycycline and immunosuppressive doses of prednisone were administered concurrently for RMSF and potential immune-mediated disease. Clinical and hematologic abnormalities resolved, and treatment was transitioned from prednisone to cyclosporine due to adverse side effects. Cyclosporine was discontinued in January 2019 and serial monthly CBCs remained normal through March 2019. When rechecked on August 9, 2019, for joint pain, hematocrit and platelet count were normal, but hypoproteinemia, hypoalbuminemia, and hypocalcemia were detected. By August 30, 2019, the dog’s anemia and thrombocytopenia worsened, despite treatment with doxycycline and prednisone. Marked abdominal effusion was documented by abdominal ultrasound, without evidence of an intra-abdominal mass. Prednisone and mycophenolate were administered for presumptive immune-mediated thrombocytopenia, and within 3 weeks, the platelet count normalized and titers increased by 4-fold. Despite medical therapy for PLN, nephrotic syndrome developed, and the dog was euthanized.

We obtained identical Rickettsia DNA gene sequences from each dog’s blood specimen. We confirmed novel Rickettsia sp. by PCR targeting 3 genes (gltA, htrA, and ompA) and 2 intergenic spacer re-
regions (23S-5S and ompA-purC) (Table 2). *Rickettsia* amplicons were 100% identical among the 3 dogs. We amplified a larger region of the *ompA* and *gltA* genes by using 3 different quantitative PCRs from case 3. We submitted sequences from this dog’s serum samples to GenBank (accession nos. MT050445–8 and MT066187). We also used the *Rickettsia* sequences from case 3 to generate a phylogenetic tree (Table 2) based on concatenated novel *Rickettsia* sp. DNA sequences and reference *Rickettsia* spp. We generated the phylogenetic tree by using the maximum-likelihood method based on the Tamura-Nei model (Figure) (6,7). Multilocus phylogenetic analysis placed the novel *Rickettsia* sp. in a clade among SFG *Rickettsia* between the human pathogens *R. heilongjiangensis* and *R. massiliae*. We attempted cell culture isolation of the *Rickettsia* sp. from whole blood but were unsuccessful (Appendix, https://wwwnc.cdc.gov/EID/article/26/12/20-0272-App1.pdf).

**Conclusions**

We report similar illnesses among 3 dogs from different US states associated with tick exposures occurring in summer months. All 3 cases demonstrated fever, lethargy, and thrombocytopenia, abnormalities commonly associated with RMSF. Case 1 had a typical acute onset fever and rapidly responded to treatment with doxycycline; case 2 had a neutrophilic polyarthritis, which has been associated with RMSF in dogs (8). Case 3 was examined for acute onset febrile illness 1 year before the novel *Rickettsia* sp. infection was documented; *Rickettsia* IFA seroreactivity was documented on both occasions. This dog likely had an unidentified, concurrent disease process that contributed to PLN.

The cases were geographically distributed among 4 states; the dogs resided in Illinois, Oklahoma, and Tennessee, but the dog from Illinois had traveled to a tick-infested area of Arkansas. The tick species were not identified, but ticks common to these states include *Amblyomma americanum*, *Dermacentor variabilis*, and *Rhipicephalus sanguineus* sensu lato, all of which are known to transmit *Rickettsia* (3). *Haemaphysalis longicornis*, an invasive tick species recently confirmed in the United States, including in Tennessee and Arkansas, should be considered a potential vector for *Rickettsia* spp. (9,10).

Based on serologic cross-reactivity, presence of *ompA*, and phylogenetic tree analysis, the new *Rickettsia* sp. is an SFG *Rickettsia*, phylogenetically related to human pathogenic *R. heilongjiangensis* and *R. massiliae*, with only 95% identity to each (11,12). Thus, we report a previously unknown and unique *Rickettsia* sp. with clinical significance for dogs and potentially humans. Because this novel *Rickettsia* cross-reacts with *R. rickettsii* on IFA, it could be underdiagnosed and more geographically widespread. Studies aimed at identifying the tick vector, potential animal reservoirs, and prevalence are ongoing. These 3 canine rickettsioses cases underscore the value of dogs as sentinels for emerging tickborne pathogens (13,14).

**Acknowledgments**

We thank Brad L. Fry for providing the clinical and diagnostic information for case 1 in this manuscript.

B.A.Q. is co-director of the North Carolina State Vector Borne Disease Diagnostic Lab (NC State VBDDL); IDEXX Laboratories (https://www.idexx.com) funds a portion of her salary. E.B.B. codirects the NC State VBDDL and the Intracellular Pathogens Research Laboratory at NC State, is chief scientific officer at Galaxy Diagnostics (https://www.galaxydx.com), and is a paid consultant for IDEXX Laboratories, Inc. No other authors have competing interests to declare.

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Appendix

Molecular Diagnostic Testing

EDTA-anticoagulated whole blood was submitted to the North Carolina State College of Veterinary Medicine, Vector-Borne Disease Diagnostic Laboratory (VBDDL) for vector-borne disease PCR testing. DNA was extracted from 200 μL aliquots of whole blood by using a QIAsymphony SP robot (QIAGEN, https://www.qiagen.com) and QIAsymphony DNA Mini Kit (QIAGEN). All sets of extractions included negative extraction controls of molecular-grade water. DNA was stored at −20°C before PCR testing. The absence of PCR inhibitors was demonstrated by an internal control quantitative PCR (qPCR) designed to amplify the host glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (1). Samples were tested for Rickettsia spp. DNA by using Rickettsia-specific PCR assays targeting 6 different DNA regions (Appendix Table). All PCR assays were run with negative molecular-grade water, a negative control of known uninfected canine DNA, and a DNA–positive control extracted from R. conorii and R. rickettsia cultures. Amplification assays were performed in CFX96 Real-Time Detection System combined with C1000 Thermal Cycler (Bio-Rad, https://www.bio-rad.com) for qPCR and an Eppendorf Mastercycler EPgradient with aluminum block for cPCR. Amplification reactions contained 12.5 μL SYBR Green Supermix (Bio-Rad) for qPCRs or MyTaq HS Mix (2×) (Bioline, https://www.bioline.com) for cPCRs, 5 μL of DNA template, primers at final concentration of 0.4 μMol and molecular-grade water to a final volume of 25 μL. We provide details on PCR reaction conditions and Rickettsia gene targets and references for PCRs we did not develop (2–6) (Appendix Table).
**Rickettsia rickettsii IFA Serologic Testing**

Serial, 2-fold dilutions of canine serum samples were made in PBS solution containing 0.05% Tween 20, 0.5% non-fat dry milk, and 1% normal goat serum (GIBCO, Fisher Scientific, https://www.fishersci.com) before adding 8–10 µL to slide wells prepared with *R. rickettsia*, originally isolated from a naturally infected dog, cultured in DH82 cells. Slides containing serial diluted canine serum was incubated in a humidified chamber at 37°C for 30 minutes and washed in PBS at room temperature at 300 rpm for 30 minutes. Slides were then air-dried before adding 8–10 µL of a 0.01 mg/mL solution of fluorescein isothiocyanate (FITC) goat anti-dog immunoglobulin G (H&L) conjugate (Sigma, https://www.sigmaaldrich.com) to each well. Slides were then incubated in a humidified chamber at 37°C for 30 min before being washed in ≈400 mL PBS at room temperature, in the dark, at 300 rpm for 20 min. Slides were washed for an additional 20 min after adding 4–5 drops of a Tween-20. Slides were then rinsed with deionized water and dried in the dark before adding a coverslip with antifading mounting medium, Vectashield (Vector Laboratories, https://vectorlabs.com). Slides were evaluated by using a ZEISS Axio Lab.A1 fluorescence microscope with exciter and barrier filters (Carl Zeiss Microscopy, https://www.zeiss.com) under ×400 magnification. Each slide contained canine seroreactive *R. rickettsia* positive control serum and canine nonreactive negative control serum. Canine serum samples were screened at 1:16, 1:32 and 1:64 dilutions, and all serum samples reactive at a titer of 1:64 were repeated and diluted to an endpoint titer of 1:8,192. To avoid confusion with possible nonspecific binding found at low dilutions, a cutoff titer of ≥1:64 was used to define a seroreactive titer.

**Rickettsia Culture**

For cases 1 and 2, we attempted to grow the new *Rickettsia* sp. in DH82 canine cells with a modified protocol (7). EDTA-anticoagulated whole blood (0.5 mL) was added to a 95% confluent monolayer of DH82 canine cells in a T-10 flask with 1 mL of media (RPMI/10% FBS). The flask was rocked slowly at room temperature for 1 hour before adding 3 mL of fresh media (RPMI/10% FBS) and placing in a 37°C incubator with 5% CO2. After 12 hours of incubation, all nonadherent content was added to a T-25 flask of 85% confluent DH82 cells. Fresh media was added to both flasks and placed in a 37°C incubator with 5% CO2. After 12
hours of incubation, the contents of both T-10 and T-25 flasks were combined into a T-75 flask, and fresh media was added to the used T-10 and T-25 flasks. Content from all flasks were incubated for 2 weeks and periodically tested by *Rickettsia* qPCR and Diff-Quick staining (RAL Diagnostics, https://www.ral-diagnostics.fr) to identify *Rickettsia* organisms.

**Sequencing and Phylogenetic Analyses**

Nucleotide sequencing of amplicons was performed by Genewiz, Inc. (https://www.genewiz.com) by using both forward and reverse primers for each DNA target. DNA sequences were analyzed by using the BLAST search algorithm and the NCBI nucleotide database and sequence alignments were performed by using Geneious Prime (https://www.geneious.com) for Java version 11.0.2+7. Sequences were deposited into NCBI nucleotide database. A total of 2,576 aligned nucleotides from regions within 3 genes (*ompA*, *gltA*, and 17 kDa) and 2 intergenic spacers (23S-5S and *mmpA-purC*), were concatenated and a multilocus phylogenetic tree was assembled by using maximum-likelihood method and Tamura-Nei model through PhyML 3.3.20180621 and Geneious Prime software (8,9).

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**Appendix Table.** Quantitative and conventional PCR assay conditions, PCR targets, primers, and associated references used in an investigation of novel *Rickettsia* in dogs, USA

| Gene target (reference) | Primers | Primer Sequence (5′–3′) | Primer, µMol | PCR product, bp | Polymerase | A/D | D | A | E | No. DAE cycles | Final E |
|------------------------|---------|--------------------------|--------------|----------------|------------|-----|---|---|---|----------------|---------|
| 23s-5s ITS (2)         | Rick23- 5_F2 | AGC TCG ATT GAT TTA CTT TGC TG | 0.4          | 215 | 98°C, 180 s | 98°C, 15 s | 62°C, 15 s | 72°C, 20 s | 40 | NA             |
|                        | Rick23- 5_R  | CCA CCA AGC TAG CAA TAC AAA | 0.4          |             |            |      |    |    |    |                 |         |
| OmpA_1† (3)            | OmpA 107F | GCT TTA TTC ACC ACC TCA AC | 0.4          | 137 | 95°C, 120 s | 95°C, 10 s | 58°C, 10 s | 72°C, 10 s | 50 | 72°C, 30 s     |
|                        | OmpA 299R | TRA TCA CCA CCC TAA GTA AAT | 0.4          |             |            |      |    |    |    |                 |         |
| OmpA_2 (4)             | Rr190.54 7F | CCT GCC GAT AAT TAT ACA GGT TTA | 0.4          | 115 | 98°C, 180 s | 98°C, 15 s | 60°C, 15 s | 72°C, 20 s | 40 |               |
|                        | Rr190.70 1R | GTT CCG TTA ATG GCA GCAT | 0.4          |             |            |      |    |    |    |                 |         |
| OmpA_3 (4)             | OmpA 107F | GCT TTA TTC ACC ACC TCA AC | 0.4          | 533 | 95°C, 120 s | 95°C, 10 s | 60°C, 10 s | 72°C, 10 s | 50 | 72°C, 30 s     |
|                        | Rr190.70 1R | GTT CCG TTA ATG GCA GCAT | 0.4          |             |            |      |    |    |    |                 |         |
| 17kDa                  | Rck 17kDa F | GCG CAT TAC TTG GTC TCTCA | 0.4          | 173 | 98°C, 180 s | 98°C, 15 s | 60°C, 15 s | 72°C, 15 s | 40 | NA             |
|                        | Rck 17kDa R | GTA GAA TGG CGT AAT CCG GA | 0.4          |             |            |      |    |    |    |                 |         |
| mmpA-purC ITS† (5)     | mppA F   | CAA ATG GCT CAA GAG AAA AA | 0.4          | 507 | 95°C, 120 s | 95°C, 15 s | 60°C, 15 s | 72°C, 30 s | 40 | 72°C, 60 s     |
|                        | mppA R   | TTT TCA ATG CCG ATC ATT TC | 0.4          |             |            |      |    |    |    |                 |         |
| GltA_1†                 | GltA F   | TGC GGA AGC CGA TTG CTT TAC | 0.4          | 847 | 95°C, 120 s | 95°C, 10 s | 58°C, 10 s | 72°C, 10 s | 50 | 72°C, 30 s     |
|                        | GltA R   | AGC TGC CCG AGT TCC TTT AAT AC | 0.4          |             |            |      |    |    |    |                 |         |
| GltA_2                  | GltA F2  | CAG TAC TTA AAG AAA CGT GCA AAG | 0.4          | 222 | 98°C, 180 s | 98°C, 15 s | 57°C, 15 s | 72°C, 20 s | 40 | NA             |
|                        | CS-6     | AGG GTC TCT GCG CAT TTC TT | 0.4          |             |            |      |    |    |    |                 |         |
| GltA_3 (6)              | CS-5     | GAG AGA AAA TTA TAT ATC CAA ATG TGG AT | 0.4          | 99  | 98°C, 180 s | 98°C, 15 s | 57°C, 15 s | 72°C, 20 s | 40 | NA             |

* A, annealing; A/D, activation/denaturation; D, denaturization; DAE, denaturation-annealing-extension; E, extension.
† Conventional PCR.