HHS Public Access

Author manuscript

*Ticks Tick Borne Dis*. Author manuscript; available in PMC 2021 July 01.

Published in final edited form as:

*Ticks Tick Borne Dis*. 2020 July ; 11(4): 101422. doi:10.1016/j.ttbdis.2020.101422.

# *Rickettsia* and *Anaplasma* species in *Dermacentor andersoni* ticks from Washington

**Lily Francis**[a], **Christopher D. Paddock**[a], **Elizabeth A. Dykstra**[b], **Sandor E. Karpathy**[a,*]

[a]Rickettsial Zoonoses Branch, National Center for Emerging and Zoonotic Infectious Disease, Centers for Disease Control and Prevention, Atlanta, GA, United States

[b]Zoonotic Disease Program, Washington State Department of Health, Olympia, WA, United States

## Abstract

*Dermacentor andersoni*, the Rocky Mountain wood tick, occurs predominantly in the northwestern United States and southwestern Canada. There are relatively few contemporary data to evaluate the occurrence of *Rickettsia* and *Anaplasma* species in *D. andersoni* in western North America, and even less information about these associations in the state of Washington, where this tick species is widely distributed and often bites humans. We used PCR assays to detect DNA of *Rickettsia* and Anaplasmataceae bacteria in 203 adult *D. andersoni* ticks collected from 17 sites in 9 counties of Washington between May 2012 and May 2015. Of these, 56 (27.6 %) were infected with a *Rickettsia* species and 3 (5.4 %) with a member of the Anaplasmataceae family. *Rickettsia peacockii*, *R. bellii* and *R. rhipicephali* were found in 17.7 %, 4.9 %, and 4.4 % of the *Rickettsia* positive ticks, respectively. Coinfections of *R. bellii* with *R. peacockii* or *R. rhipicephali* were identified in 6 ticks. Of the Anaplasmataceae-positive ticks, one was identified as being infected with *Anaplasma phagocytophilum* AP-Variant 1. No ticks were infected with a recognized human or animal pathogen, including *R. rickettsii, A. phagocytophilum*-ha, *A. bovis*, or *A. marginale*.

### Keywords

Dermacentor andersoni; Rickettsia; Anaplasma phagocytophilum

## 1. Introduction

*Dermacentor andersoni*, the Rocky Mountain wood tick, is found predominantly in the northwestern United States and southwestern Canada (Chapman et al., 2006). Nymphal and larval *D. andersoni* feed primarily on small mammals and adult ticks feed primarily on larger animals such as deer, cattle, horses, elk, and occasionally, humans (Eisen, 2007; James et al., 2006). *Dermacentor andersoni* is a historically recognized vector of *Rickettsia*

*Corresponding author at: Centers for Disease Control and Prevention, MS H17-3, 1600 Clifton Rd., NE, Atlanta, GA, 30329, United States. States.skarpathy@cdc.gov (S.E. Karpathy).

CRediT authorship contribution statement

**Lily Francis:** Writing - original draft. **Christopher D. Paddock:** Conceptualization, Methodology, Writing - review & editing. **Elizabeth A. Dykstra:** Conceptualization, Writing - review & editing. **Sandor E. Karpathy:** Conceptualization, Methodology, Investigation, Writing - review & editing.

*rickettsii*, the agent of Rocky Mountain spotted fever (RMSF). More recently, various other rickettsial agents have been identified in *D. andersoni* ticks in the western United States and Canada, including *R. peacockii, R. bellii*, and *R. rhipicephali*, none of which have caused known disease in humans (Parola et al., 2013). Additionally, *Anaplasma bovis* and *A. marginale*, which are of unknown pathogenicity to humans but cause disease in cattle and other wild ruminants, have been identified in *D. andersoni* (Rar and Golovljova, 2011).

There are relatively few contemporary data to evaluate rickettsial agents in *D. andersoni* in western North America and even less information about *Rickettsia* and *Anaplasma* species associated with *D. andersoni* in Washington state, where this tick species is distributed broadly and frequently bites humans (Dergousoff et al., 2009; Dworkin et al., 1999; Niebylski et al., 1997; Philip et al., 1983). Previous work by Gall et al. has highlighted the importance of examining ticks collected from different geographic sites as the bacterial microbiome composition was shown to be affected by the geographic origin of *D. andersoni* ticks (Gall et al., 2016, 2017). Thus, for this study a survey of *Rickettsia* and *Anaplasma*-infected *D. andersoni* from multiple sites in Washington was conducted to provide information about potential tick-borne pathogens in those areas which can better inform healthcare providers and local residents of some of the risks associated with bites of *D. andersoni* in this region.

## 2. Methods

### 2.1. Sample collection

Two hundred and three adult-stage *D. andersoni* ticks were collected from 17 sites in 9 counties (Benton, Chelan, Ferry, Klickitat, Kittitas, Lincoln, Okanogan, Spokane, and Yakima) of Washington between May 2012 and May 2015 and were identified using a standard taxonomic key (Brinton et al., 1965). The ticks were collected by several methods, including, dragging, flagging, and direct removal from the clothing of collectors while flagging or dragging; of these, 105 were female and 98 were male (Table 1). The ticks were stored in 70 % ethanol until DNA was extracted.

### 2.2. DNA extraction and molecular evaluation

Ticks were minced individually using sterile scalpels and DNA was extracted using QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, Valencia, California) following the manufacturer's recommendations. Samples were eluted in a final volume of 100 μL and stored at 4 °C until polymerase chain reaction (PCR) analyses were performed. Samples were screened using a pan-*Rickettsia* real-time PCR assay (Kato et al., 2013). Samples were screened in duplicate and a negative control consisting of sterile water in place of template DNA and a positive control consisting of *R. conorii* DNA were included on every plate. Samples with a $C_q < 40$ were considered positive. Positive samples were tested for DNA of spotted fever group *Rickettsia* species using a semi-nested *omp*A assay (Eremeeva et al., 2006). Amplicons of the expected size (532 bp) were sequenced in both directions on an Applied Biosystems 3500 Genetic Analyzer and the reads were assembled using Geneious v10.2.2. All pan-*Rickettsia* positive samples were also screened using a *R. bellii*-specific real-time PCR assay in which both the primer pair and the probe were designed to be highly

specific for *R. bellii* (Hecht et al., 2016; Szabo et al., 2013). Samples with a $C_q < 40$ were considered positive for *R. bellii* infection. DNA samples were also tested by using a real-time pan-Anaplasmataceae assay targeting the 16S rRNA gene (Eremeeva et al., 2007; Li et al., 2002). Amplicons (146–154 bp) were gel purified, sequenced, and analyzed as previously described to determine the bacterial species.

## 3. Results

Of the 203 ticks screened using the pan-*Rickettsia* and Anaplasmataceae assays, 56 (27.6 %) contained DNA of a *Rickettsia* sp. and three (5.4 %) contained DNA of a member of the Anaplasmataceae family (Table 2). *Rickettsia peacockii* infections were the most prevalent at 17.7 % (n = 36); sequences were 98.4 %–100 % identical to *R.peacockii* strain 5 (GenBank KJ675444). *Rickettsia bellii* was the next most prevalent rickettsial species identified, with 4.9 % (n = 10) of the ticks positive for DNA from this species. *Rickettsia rhipicephali* was identified in 4.4 % (n = 9) of the tick samples; all nine sequences were identical to each other and were 99.4 % identical to strain Do276 (GenBank EU109175). The AP-Variant 1 genotype of *A. phagocytophilum* (100 % identical to AP-Variant 1 isolate CRT38; GenBank APHI000002.1) was identified in 1 tick (0.5 %) while 2 ticks (0.9 %) were infected with undetermined Anaplasmataceae. PCR analysis revealed 10 ticks positive for *R. bellii*; six of these were co-infections with either *R. peacockii* (5) or *R. rhipicephali* (1). Seven of the *Rickettsia*-infected samples and two of the Anaplasmataceae-infected samples had high $C_q$ values and the DNA concentrations were too low to sequence for species confirmation.

Each of the Anaplasmataceae-infected ticks were collected at different sites in Chelan, Lincoln, and Spokane counties, while a majority of the *Rickettsia*-infected ticks (34 ticks, 60.7 %) were collected from two sites in Lincoln County.

## 4. Discussion

We identified three distinct *Rickettsia* species and an *A. phagocytophilum* variant among approximately 200 questing adult *D. andersoni* collected from 9 counties of Washington. Among these, *R. peacockii* was the most commonly identified agent. To our knowledge, we identified for the first time co-infections of *D. andersoni* comprising *R. bellii* and *R. peacockii*, and those comprising *R. bellii* and *R. rhipicephali* from Washington. Although uncommon, co-infections (especially those involving *R. bellii*) with multiple rickettsial species have been previously reported (Gall et al., 2017; Hecht et al., 2019; Paddock et al., 2018; Wikswo et al., 2008; Wright et al., 2015). We also identified a variant of *A. phagocytophilum* in *D. andersoni*, which has not been reported previously. Nonetheless, we did not detect any recognized human or animal pathogens described previously in *D. andersoni*, including *R. rickettsii, A. marginale*, or *A. bovis*

*Rickettsia peacockii* was originally identified in *D. andersoni* in Western Montana where 66.1 % of the collected ticks were infected (Niebylski et al., 1997). The high prevalence of *R. peacockii* in Western Montana and the provinces of Alberta and Saskatchewan (76 %) in Canada is similar to the relatively high percentage of *D. andersoni* infected with *R.*

*peacockii* in Washington (Dergousoff et al., 2009). There is some evidence that indicates rickettsial infection within a vector inhibits transmission of a second *Rickettsia* (Macaluso et al., 2002). This has been described specifically in *D. andersoni* infected with *R. peacockii* inhibiting the transmission of *R. rickettsii* (Burgdorfer et al., 1981). It is possible that the relatively high prevalence of *R. peacockii* could be responsible for the absence of *R. rickettsii* from the sample set, but analysis of progeny of *R. peacockii*-infected ticks is necessary to confirm this hypothesis (Hayes and Burgdorfer, 1989).

The AP-Variant 1 genotype of *A. phagocytophilum* was identified; however, this variant is not believed to infect humans (Massung et al., 2006). *Dermacentor andersoni* is a known vector for *A. bovis* and *A. marginale* (Rar and Golovljova, 2011). *Anaplasma marginale* has been detected in infected calves from the same county as one of the sites where samples were collected in Washington (Palmer et al., 1989). Additionally, although no tick vector was identified, *A. phagocytophilum* has been identified in canines in Washington (Poitout et al., 2005).

Because only one tick was collected from many sites, data may be non-representative of these sites, county, or state as a whole. This precludes any estimation of frequency based on the findings of this study. However, these preliminary data suggest that rickettsial pathogens are infrequent among *D. andersoni* ticks encountered in Washington. While other researchers have found high frequency of rickettsial pathogens in *D. andersoni* in the western United States, ticks have specific ecological needs that determine their distribution (Niebylski et al., 1997). In a study from Bitterroot Valley, Montana, it was found that *D. andersoni* on one side of the valley were not infected with *R. peacockii* while a majority of *D. andersoni* collected from the other side of the valley were infected (Philip and Casper, 1981). These isolated populations seemed not to interact or transmit pathogens between each other. Additionally, animals may serve both as blood meals for the ticks and as reservoirs for the bacterial pathogens. Thus, the presence or absence of specific animal species may also play a role in determining the presence or absence of a particular rickettsial agent in the tick population (Burgdorfer et al., 1966; Eremeeva and Dasch, 2015; Parola et al., 2005). Because of trends like this, the small sample size and convenience sampling in this study are a limiting factor in defining *Rickettsia* and *Anaplasma* in Washington's *D. andersoni* populations.

## 5. Conclusion

A total of 203 adult *D. andersoni* ticks from Washington were analyzed for the presence of rickettsial disease. The most prevalent *Rickettsia* species was *R. peacockii*, a non-pathogenic bacterium that could inhibit the transmission of the etiological agent of RMSF, *R. rickettsii*. Other *Rickettsia* species found in the samples included *R. rhipicephali* and *R. bellii*, although neither have been definitively linked with disease in humans. One tick was infected with a non-pathogenic variant of *A. phagocytophilum*. These results suggest that the frequency of medically important rickettsial pathogens in *D. andersoni* ticks in Washington state is low.

## Acknowledgments

The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

## References

Brinton EP, Beck DE, Allred DM, 1965 Identification of the adults, nymphs and larvae of ticks of the genus Dermacentor Koch (Ixodidae) in the western United States. Brigham Young Univ. Sci. Bull. Biol. Ser 5 (4), 1.

Burgdorfer W, Friedhoff KT, Lancaster JL Jr., 1966 Natural histroy of tick-borne spotted fever in the USA. Bull. World Health Organ 35 (2), 149–153. [PubMed: 5296999]

Burgdorfer W, Hayes SF, Mavros AJ, 1981 Nonpathogenic rickettsiae in Dermacentor andersoni: a limiting factor for the distribution of Rickettsia rickettsia In: Burgdorfer W, Anacker RL (Eds.), Rickettsiae and Rickettsial Diseases. Academic Press, New York, NY, pp. 585–594.

Chapman AS, Bakken JS, Folk SM, Paddock CD, Bloch KC, Krusell A, Sexton DJ, Buckingham SC, Marshall GS, Storch GA, Dasch GA, McQuiston JH, Swerdlow DL, Dumler SJ, Nicholson WL, Walker DH, Eremeeva ME, Ohl CA, 2006 Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis—United States: a practical guide for physicians and other health-care and public health professionals. MMWR Recomm Rep. 55 (RR-4), 1–27.

Dergousoff SJ, Gajadhar AJ, Chilton NB, 2009 Prevalence of Rickettsia species in Canadian populations of Dermacentor andersoni and D. variabilis. Appl. Environ. Microbiol 75 (6), 1786–1789. [PubMed: 19151178]

Dworkin MS, Shoemaker PC, Anderson DE Jr., 1999 Tick paralysis: 33 human cases in Washington state, 1946–1996. Clin. Infect. Dis 29, 1435–1439. [PubMed: 10585792]

Eisen L, 2007 Seasonal pattern of host-seeking activity by the human-biting adult life stage of Dermacentor andersoni (Acari: Ixodidae). J. Med. Entomol 44 (2), 359–366. [PubMed: 17427709]

Eremeeva ME, Dasch GA, 2015 Challenges posed by tick-borne rickettsiae: eco-epidemiology and public health implications. Front. Publ. Health 3, 55.

Eremeeva ME, Bosserman EA, Demma LJ, Zambrano ML, Blau DM, Dasch GA, 2006 Isolation and identification of Rickettsia massiliae from Rhipicephalus sanguineus ticks collected in Arizona. Appl. Environ. Microbiol 72 (8), 5569–5577. [PubMed: 16885311]

Eremeeva ME, Oliveira A, Moriarity J, Robinson JB, Tokarevich NK, Antyukova LP, Pyanyh VA, Emeljanova ON, Ignatjeva VN, Buzinov R, Pyankova V, Dasch GA, 2007 Detection and identification of bacterial agents in Ixodes persulcatus Schulze ticks from the north western region of Russia. Vector Borne Zoonotic Dis 7 (3), 426–436. [PubMed: 17767409]

Gall CA, Reif KE, Scoles GA, Mason KL, Mousel M, Noh SM, Brayton KA, 2016 The bacterial microbiome of Dermacentor andersoni ticks influences pathogen susceptibility. ISME J 10 (8), 1846–1855. [PubMed: 26882265]

Gall CA, Scoles GA, Magori K, Mason KL, Brayton KA, 2017 Laboratory colonization stabilizes the naturally dynamic microbiome composition of field collected Dermacentor andersoni ticks. Microbiome 5 (1), 133. [PubMed: 28978338]

Hayes SF, Burgdorfer W.J.Iem., 1989 Physiology, Genetics, Evolution. CRC Press, Boca Raton, Fla, pp. 235–251 Interactions between rickettsial endocytobionts and their tick hosts.

Hecht JA, Allerdice MEJ, Krawczak FS, Labruna MB, Paddock CD, Karpathy SE, 2016 Development of a Rickettsia bellii-specific TaqMan assay targeting the citrate synthase gene. J. Med. Entomol 53 (6), 1492–1495. [PubMed: 27473178]

Hecht JA, Allerdice MEJ, Dykstra EA, Mastel L, Eisen RJ, Johnson TL, Gaff HD, Varela-Stokes AS, Goddard J, Pagac BB, Paddock CD, Karpathy SE, 2019 Multistate survey of American Dog ticks (Dermacentor variabilis) for Rickettsia species. Vector Borne Zoonotic Dis 19 (9), 652–657. 10.1089/vbz.2018.2415. [PubMed: 30942664]

James AM, Freier JE, Keirans JE, Durden LA, Mertins JW, Schlater JL, 2006 Distribution, seasonality, and hosts of the Rocky Mountain wood tick in the United States. J. Med. Entomol 43 (1), 17–24. [PubMed: 16506443]

Kato CY, Chung IH, Robinson LK, Austin AL, Dasch GA, Massung RF, 2013 Asessment of real-time PCR assay for detection of Rickettsia spp. and Rickettsia rickettsii in banked clinical samples. J. Clin. Microbiol 51 (1), 314–317. [PubMed: 23135935]

Li J.S. y., Chu F, Reilly A, Winslow GM, 2002 Antibodies highly effective in SCID mice during infection by the intracellular bacterium Ehrlichia chaffeensis are of pi-comolar affinity and exhibit preferential epitope and isotype utilization. J. Immunol 169, 1419–1425. [PubMed: 12133967]

Macaluso KR, Sonenshine DE, Ceraul SM, Azad AF, 2002 Rickettsial infection in Dermacentor variabilis (Acari: Ixodidae) inhibits transovarial transmission of a second Rickettsia. J. Med. Entomol 39 (6), 809–813. [PubMed: 12495176]

Massung RF, Mather TN, Levin ML, 2006 Reservoir competency of goats for the Ap-variant 1 strain of Anaplasma phagocytophilum. Infect. Immun 74 (2), 1373–1375. [PubMed: 16428787]

Niebylski ML, Schrumpf ME, Burgdorfer W, Fischer ER, Gage KL, Schwan TG, 1997 Rickettsia peacockii sp. nov., a new species infecting wood ticks, Dermacentor andersoni, in Western Montana. Int. J. Syst. Bacteriol 47 (2), 446–452. [PubMed: 9103635]

Paddock CD, Yoshimizu MH, Zambrano ML, Lane RS, Ryan BM, Espinosa A, Hacker JK, Karpathy SE, Padgett KA, 2018 Rickettsia species isolated from Dermacentor occidentalis (Acari: Ixodidae) from California. J. Med. Entomol 55 (6), 1555–1560. 10.1093/jme/tjy100. [PubMed: 29982649]

Palmer GH, Barbet AF, Cantor GH, McGuire TC, 1989 Immunization of cattle with the MSP-1 surface protein complex induces protection against a structurally variant Anaplasma marginale isolate. Infect. Immun 57 (11), 3666–3669. [PubMed: 2807542]

Parola P, Paddock CD, Raoult D, 2005 Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin. Microbiol. Rev 18 (4), 719–756. [PubMed: 16223955]

Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, Abdad MY, Stenos J, Bitam I, Fournier PE, Raoult D, 2013 Update on tick-borne rickettsioses around the world: a geographic approach. Clin. Microbiol. Rev 26(4), 657–702. [PubMed: 24092850]

Philip RN, Casper EA, 1981 Serotypes of spotted fever group rickettsiae isolated from Dermacentor andersoni (Stiles) ticks in western Montana. Am. J. Trop. Med. Hyg 30(1), 230–238. [PubMed: 6782899]

Philip RN, Casper EA, Anacker RL, Cory J, Hayes SF, Burgdorfer W, Yunker CE, 1983 Rickettsia bellii sp. nov.: a tick-borne Rickettsia, widely distributed in the United States, that is distinct from the spotted fever and typhus biogroups. Int. J. Syst. Bacteriol 33 (1), 94–106.

Poitout FM, Shinozaki JK, Stockwell PJ, Holland CJ, Shukla SK, 2005 Genetic variants of Anaplasma phagocytophilum infecting dogs in Western Washington State. J. Clin. Microbiol 43 (2), 796–801. [PubMed: 15695682]

Rar V, Golovljova I, 2011 Anaplasma, Ehrlichia, and “Candidatus Neoehrlichia” bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. Infect. Genet. Evol 11 (8), 1842–1861. [PubMed: 21983560]

Szabo MP, Nieri-Bastos FA, Spolidorio MG, Martins TF, Barbieri AM, Labruna MB, 2013 In vitro isolation from Amblyomma ovale (Acari: Ixodidae) and ecological aspects of the Atlantic rainforest Rickettsia, the causative agent of a novel spotted fever rickettsiosis in Brazil. Parasitology 140 (6), 719–728. [PubMed: 23363571]

Wikswo ME, Hu R, Dasch GA, Krueger L, Arugay A, Jones K, Hess B, Bennett S, Kramer V, Eremeeva ME, 2008 Detection and identification of spotted fever group rickettsiae in Dermacentor species from southern California. J. Med. Entomol 45 (3), 509–516. [PubMed: 18533446]

Wright CL, Sonenshine DE, Gaff HD, Hynes WL, 2015 Rickettsia parkeri transmission to Amblyomma americanum by cofeeding with Amblyomma maculatum (Acari: Ixodidae) and potential for spillover. J. Med. Entomol 52 (5), 1090–1095. [PubMed: 26336226]

**Table 1**

Collection location and source of *Dermacentor andersoni* ticks collected in Washington and evaluated by PCR for *Rickettsia* and *Anaplasma* species.

| County | Collection Site | Source | No. Tested |
|---|---|---|---|
| Benton | 46.22374, − 119.28775 | Host check | 1 |
| Chelan | 47.56564, − 120.78279 | Hand collection/host check | 2 |
| Ferry | 48.60055, − 118.62843 | Drag/flag | 6 |
| Klickitat | 45.69707, − 121.29003 | Drag/flag | 2 |
| Kittitas | 46.9965, − 120.5478 | Hand collection | 1 |
| Lincoln | 47.7585, − 118.5197 | Host check | 52 |
| | 47.29700, − 120.75960 | Host check | 1 |
| Okanogan | 48.9391, − 119.4356 | Hand collection | 1 |
| | 48.7052, − 119.4395 | Host check | 7 |
| Spokane | 47.40705, − 117.58925 | Drag | 5 |
| | 47.4874, − 117.5758 | Host check | 3 |
| | 47.7443, − 117.0688 | Hand collection | 1 |
| | 47.60825, − 117.05212 | Host check | 1 |
| | 47.77628, − 117.5468 | Hand collection | 1 |
| | 47.41386, − 117.59376 | Drag/hand collection | 115 |
| Yakima | 46.91500, − 121.05230 | Host check | 1 |
| | 46.5351, − 120.8673 | Host check | 1 |

**Table 2**

Results of PCR analysis for the detection of *Rickettsia* or Anaplasmataceae in 203 *D. andersoni*.

| Agent | No. (% of 203) | No. Male (% of 98) | No. Female (% of 105) |
|---|---|---|---|
| *Rickettsia spp.* | 56 (27.6 %) | 24 (24.5 %) | 32 (30.5 %) |
| *R. peacockii* only | 31 (15.3 %) | 12 (12.2 %) | 19 (18.1 %) |
| *R. peacockii* and *R. bellii* | 5 (2.5 %) | 3 (3.1 %) | 2 (1.9 %) |
| *R. rhipicephali* only | 8 (3.9 %) | 2 (2.0 %) | 6 (5.7 %) |
| *R. rhipicephali* and *R. bellii* | 1 (0.5 %) | 1 (1.0 %) | 0 (0.00 %) |
| *R. bellii* only | 4 (2.0 %) | 3 (3.1 %) | 1 (1.0 %) |
| Anaplasmataceae | 3 (5.4%) | 3 (3.1 %) | 0 (0.00 %) |