Prevalence of Seropositivity to Spotted Fever Group Rickettsiae and Anaplasma phagocytophilum in a Large, Demographically Diverse US Sample

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Background. Most epidemiologic studies of tick-borne rickettsial diseases in the United States are small and have limited demographic scope, making broader risk assessment difficult.

Methods. We conducted a seroprevalence study of spotted fever group rickettsiae and Anaplasma phagocytophilum, the agent of human granulocytic anaplasmosis. Specimens were selected randomly from the Department of Defense Serum Repository for 10,000 diverse military personnel at various stages in their careers who were serving with active duty status in 1997. Antibody testing included enzyme-linked immunosorbent assay for Rickettsia rickettsii and A. phagocytophilum, and Western blot confirmation for A. phagocytophilum. Risk factors were assessed using logistic regression.

Results. Subjects were mostly male and young and were diverse ethnically and geographically. Spotted fever group rickettsiae seropositivity was 6.0% (95% confidence interval, 5.5%–6.4%). In univariable logistic regression, seroprevalence was significantly higher among older subjects, men (6.5%, compared with 3.3% among women), black individuals (8.7%, compared with 5.6% among white individuals), subjects from states with above-average Rocky Mountain spotted fever incidence, and subjects in ground combat specialties. Associations remained significant in multivariable analysis for age, sex, black versus white race, home state with high incidence, and ground combat specialty. Among 696 subjects with serum samples obtained within 7 days after entering the military, the rate of seropositivity was 3.4% (95% confidence interval, 2.1%–4.8%). Seroprevalence was nonsignificantly lower in men (3.4%, compared with 3.7% in women) and in black individuals (3.4%, compared with 4.1% in white individuals). A. phagocytophilum seropositivity, as determined by enzyme-linked immunosorbent assay and Western blot, was 2.6% and 0.11% (95% confidence interval, 0.05%–0.18%), respectively. Western blot seropositivity was not significantly associated with subject characteristics in univariable analysis.

Conclusions. Spotted fever group rickettsiae exposure was common and A. phagocytophilum exposure was rare in a US population with broad demographic diversity.

Rocky Mountain spotted fever (RMSF) and human granulocytic anaplasmosis (HGA) are nationally notifiable tick-borne rickettsial diseases in the United States. However, significant underreporting to the Centers for Disease Control and Prevention is suspected for both diseases, because symptoms can be nonspecific and commonly used diagnostic tests are not highly accurate [1, 2].

The agent of RMSF, Rickettsia rickettsii, is a small, gram-negative, obligate intracellular bacterium that infects endothelial and smooth muscle cells. R. rickettsii is transmitted by Demacentor variabilis (American dog tick) and Demacentor andersonii (Rocky Mountain wood tick) in the eastern and western United States, respectively. Acute disease typically manifests as a nonspecific febrile syndrome, followed by a rash that progresses from macules to the petechial form from which the name derives. RMSF improves rapidly with doxycycline treatment, which is the Centers for Disease Control and Prevention–recommended first-line treatment.

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for presumptive or confirmed tick-borne rickettsial diseases [3]. The case-fatality rate has been estimated at 1.4% [1]; delay in treatment is strongly associated with fatal outcome [4]. RMSF incidence was estimated at 2.2 cases per 1 million population per year in the United States during the period 1997–2002 on the basis of surveillance data, with cases in all but 2 of the continental 48 states and with 5 states (Tennessee, Oklahoma, Arkansas, North Carolina, and South Carolina) reporting more than one-half of the cases [1]. Recently, *Rickettsia parkeri* became the second tick-borne spotted fever group (SFG) rickettsiosis identified in the United States [5–7]. Because commonly used diagnostic tests for RMSF do not distinguish among SFG rickettsiae, implication of *R. parkeri* in human disease has supported speculation that this or another SFG rickettsiae may be responsible for some cases diagnosed as RMSF [8].

The agent of HGA, *Anaplasma phagocytophilum*, which is also a gram-negative, obligate intracellular bacterium, infects granulocytes (the disease was previously called human granulocytic ehrlichiosis until reclassification of the agent from the genus *Ehrlichia* [9]). *A. phagocytophilum* is transmitted by *Lxodes scapularis* (black-legged deer tick) and *Lxodes pacificus* (western black-legged tick) in the eastern and western United States, respectively. Clinical symptoms vary widely but often include fever, headache, myalgia, and malaise [10]. Although death is rare (case-fatality rate, 0.5%), approximately one-half of symptomatic patients require hospitalization, and some (5%–7% of symptomatic patients) require intensive care [10, 11]. HGA incidence was estimated at 1.4 cases per 1 million population per year in the United States during the period 2001–2002 on the basis of surveillance data, with higher incidence in the Northeast, mid-Atlantic, upper Midwest, and northern California [2].

Seroprevalence studies for SFG rickettsiae and *A. phagocytophilum* exposure in the United States have been small and have focused on localized or high-risk populations. To better assess exposure across a wide geographic distribution, we conducted a cross-sectional study in a representative sample of 10,000 military personnel.

**METHODS**

**Study design.** Selection of the study population has been described elsewhere [12]. In brief, 10,000 specimens from the Department of Defense Serum Repository were randomly selected from the 1.4 million active duty service members in December 1997 (9673 of these specimens were analyzed for *Borrelia burgdorferi* exposure in Barker et al. [12]). Sampling was stratified by branch of military service to ensure representative proportions of Army, Navy, Air Force, and Marine Corps personnel. Specimens had been collected in 1997 for routine HIV screening or prior to deployment on a military mission. Samples were coded to maintain anonymity.

**Demographic and occupational data.** Anonymous subject data included age, sex, ethnicity, home of record, education level, branch of military service, length of service, rank, and military occupational specialty. Home state incidences of RMSF during the period 1997–2002 and of HGA during the period 2001–2002 were obtained from Centers for Disease Control and Prevention surveillance reports [1, 2]. Data were unavailable for some subjects for education level, home of record, length of military service, and home state incidence of HGA.

**Serological testing.** For assessment of SFG rickettsiae exposure, an ELISA was performed for all 10,000 specimens using *R. rickettsii* whole-cell antigen in microwell format. Ninety-six-well plates (Dynatech Laboratories) were coated with a 1:4000 dilution in PBS of whole-cell antigen from *R. rickettsii* R strain, prepared as described previously [13], which were incubated for 48 h at 4°C. Plates were then blocked with 200 μL of 5% nonfat milk (Difco) in wash buffer (0.01% Tween-20 in PBS) at room temperature for 1 h. After 3 washes with wash buffer, 100 μL of serum samples and control samples, diluted in 5% nonfat milk in wash buffer for final dilutions of 1:100, 1:400, 1:1600, and 1:6400, were added to corresponding wells. The samples were allowed to incubate for 1 h at room temperature. The microwells were then washed 3 times, and 100 μL of secondary antibody peroxidase-conjugated goat anti-human IgG (Kirkegaard and Perry Laboratories), diluted 1:2000 in 5% nonfat milk in wash buffer, was added to each well and incubated for 1 h at room temperature. Subsequently, the microwells were washed with wash buffer, and 100 μL of a peroxidase substrate, 2,2’-azino-di-[ethylbenzthiazoline sulfonate] (Kirkegaard and Perry Laboratories), was added. Plates were incubated at room temperature in a dark area for up to 30 min. The optical densities of the wells were read at 405–650 nm by a Vmax/Kinetic Microplate Reader (Molecular Devices). ELISA-positive titers were determined to be the inverse of the highest dilution in which a net optical density (absorbance with antigen minus absorbance without antigen) of ≥0.200 was obtained. Three negative control serum samples were evaluated with each run and consistently had absorbance readings <0.200.

A random subset of ELISA-positive specimens (83 specimens) was tested by Western blot. Serum samples were diluted 1:100 in dilution buffer and applied to nitrocellulose membrane strips to which SDS-PAGE–separated antigens (1 μg of *R. rickettsii* whole cells) had been transferred. Following 1-h incubation at 4°C and wash, the strips were treated with horseradish peroxidase conjugated with goat anti-human IgG for 1 h at 4°C. The strips were then washed, and immunoreactive bands were detected by chemiluminescence. Positive samples were those in which the 120-kDa rOmpB band and lipopolysaccharide smear were visible.

For assessment of *A. phagocytophilum* exposure, ELISA was performed for the 9987 specimens with sufficient volume using
a recombinantly expressed antigen (p44 derivative) in microwell format (Immunetics). For the assay, 1:20 dilutions of human serum samples, kit controls, and kit calibrators were added to antigen-coated microwells and incubated for 30 min at room temperature. After 3 washes with 0.01% Tween 20 in 1 × PBS, antigen-specific antibodies were detected by the addition of horseradish peroxidase-conjugated goat anti-human IgG for 20 min at room temperature. Subsequently, the microwells were washed as described above, and a chromogenic peroxidase substrate containing tetramethyl benzidine was added for 4 min. Dilute sulfuric acid was added to quench the color development, and the optical absorbance was measured in each well at 450 nm by a UV Max Kinetic Microplate Reader (Molecular Devices). A positive ELISA reaction was defined as occurring in a sample that gave a reactive index value >1.1. The reactive index was determined by the absorbance of the serum sample divided by the cutoff value. The cutoff value was determined by the mean absorbance of 3 calibrator samples divided by the conversion factor (1.1). A negative serological test result was defined as a reactive index <0.9, and an equivocal result was a sample with a reactive index >0.9 but <1.1.

All specimens with positive and equivocal results were subjected to Western blot. Serum samples were diluted 1:100 in dilution buffer (PBS pH, 7.4; 0.1% Tween 20 and 5% skim milk) and applied to nitrocellulose membrane (Bio-Rad) strips to which SDS-PAGE–separated antigens (0.065 μg A. phagocytophilum rP44 per strip) had been transferred. Following 1-h incubation at 4°C and washing, the strips were treated with horseradish peroxidase-conjugated goat anti-human IgG (Kidegaard & Perry Laboratories) for 1 h at 4°C. The strips were then washed and incubated for 30 min at room temperature with Opti-4CN (Bio-Rad) for colorimetric detection.

**Statistical analysis.** All variables were treated as categorical. Using Centers for Disease Control and Prevention surveillance data, home of record was classified according to whether the reported incidence of RMSF during the period 1997–2002 was equal to or above versus below the national incidence of 2.2 cases per 1 million population per year during that period [1] and whether the reported incidence of HGA during the period 2001–2002 was equal to or above versus below the national average of 1.4 cases per 1 million population per year during that period [2]. Associations between subject characteristics and serological results were studied using univariable and multivariable logistic regression. Military occupational specialties were classified as being ground combat or other because of the hypothesized greater risk for tick-borne disease in ground combat assignments. For multivariable analyses, backward elimination starting with the full model, including all covariates, was used until further elimination significantly worsened fit compared with the full model (P < .05, by likelihood ratio test). Statistical analyses were performed using Stata software, version 9 (Stata).

**Ethical review.** The study was reviewed by the human use review committee of the Naval Medical Research Center (Silver Spring, MD).

**RESULTS**

Subjects predominantly were young, male, white, and enlisted; were classified as having achieved high school or lower education level; and served in occupational specialties other than ground combat (table 1). They represented all 50 states, the District of Columbia, and 4 US territories.

| Table 1. Demographic and clinical characteristics of the study population. |
|-----------------------|------------------|------------------|
| Characteristic        | Study population (n = 10,000) |
| Age, mean years ± SD  | 28.2 ± 7.6 |
| Sex                   |                  |
| Male                  | 8428 (84) |
| Female                | 1572 (16) |
| Ethnicity             |                  |
| White, non-Hispanic   | 6779 (68) |
| Black, non-Hispanic   | 1989 (20) |
| Hispanic              | 1107 (11) |
| Other                 | 125 (1) |
| Education             |                  |
| High school or lower  | 6313 (63) |
| College or higher     | 3308 (33) |
| Unknown               | 379 (4) |
| RMSF incidence in home state |                  |
| <US incidence         | 6620 (66) |
| ≥US incidence         | 2088 (21) |
| Unknown               | 1292 (13) |
| HGA incidence in home state |                  |
| <US incidence         | 6537 (65) |
| ≥US incidence         | 982 (10) |
| Unknown               | 2481 (25) |
|Military rank          |                  |
| Enlisted              | 8469 (85) |
| Warrant officer       | 120 (1) |
| Officer               | 1411 (14) |
|Military service branch |                |
| Army                  | 3400 (34) |
| Navy                  | 2800 (28) |
| Air Force             | 2600 (26) |
| Marine Corps          | 1200 (12) |
|Length of military service*, mean years ± SD | 7.2 ± 6.6 |
| Military occupational specialty |            |
| Not ground combat     | 8926 (89) |
| Ground combat         | 1074 (11) |

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated. HGA, human granulocytic anaplasmosis; RMSF, Rocky Mountain spotted fever.

* Unknown for 11 subjects.
Antibodies against SFG rickettsiae were detected by ELISA in 597 (6.0%) of 10,000 subjects (95% CI, 5.5%–6.4%). Univariable logistic regression showed significant, positive associations between ELISA seropositivity and older age, male sex, black versus white ethnicity, home state of record with high RMSF incidence, longer military service, and ground combat specialty, as well as significant, negative associations between ELISA seropositivity and Hispanic versus White ethnicity and service in the Navy or Air Force versus Army (table 2). In multivariable logistic regression, ELISA seropositivity remained significantly more likely in the oldest versus youngest age quartile, male individuals versus female individuals, black individuals versus white individuals, service members from states with high RMSF incidence, and service members in ground combat specialties; ELISA seropositivity remained significantly less likely in Navy versus Army personnel (table 3). Western blot confirmed ELISA results in 76 (92%) of 83 positive serum samples randomly selected for verification.

To exclude exposures encountered during military service, we performed subgroup analysis for subjects whose serum sam-

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### Table 2. Serological results for spotted fever group rickettsiae.

| Characteristic                        | No. of samples tested | No. (%) of samples with positive ELISA results | OR* (95% CI) |
|---------------------------------------|-----------------------|-----------------------------------------------|--------------|
| **Age quartile, years**               |                       |                                               |              |
| 17–22                                 | 2501                  | 126 (5.0)                                     | Reference    |
| 23–26                                 | 2499                  | 136 (5.4)                                     | 1.1 (0.9–1.4) |
| 27–33                                 | 2500                  | 151 (6.0)                                     | 1.2 (0.9–1.5) |
| >34                                   | 2500                  | 184 (7.4)                                     | 1.5 (1.2–1.9) |
| **Sex**                               |                       |                                               |              |
| Female                                | 1572                  | 52 (3.3)                                      | Reference    |
| Male                                  | 8428                  | 545 (6.5)                                     | 2.0 (1.5–2.7) |
| **Ethnicity**                         |                       |                                               |              |
| White, non-Hispanic                   | 6779                  | 382 (5.6)                                     | Reference    |
| Black, non-Hispanic                   | 1989                  | 173 (8.7)                                     | 1.6 (1.3–1.9) |
| Hispanic                              | 1107                  | 40 (3.6)                                      | 0.6 (0.5–0.9) |
| Other                                 | 125                   | 2 (1.6)                                       | 0.3 (0.1–1.1) |
| **Education**                         |                       |                                               |              |
| High school or lower                  | 6313                  | 384 (6.1)                                     | Reference    |
| College or higher                     | 3308                  | 196 (5.9)                                     | 1.0 (0.8–1.2) |
| **RMSF incidence in home state**      |                       |                                               |              |
| <US incidence                         | 6620                  | 320 (4.8)                                     | Reference    |
| >US incidence                         | 2088                  | 194 (9.3)                                     | 2.0 (1.7–2.4) |
| **Military rank**                     |                       |                                               |              |
| Enlisted                              | 8469                  | 494 (5.8)                                     | Reference    |
| Warrant officer                       | 120                   | 12 (10.0)                                     | 1.8 (0.9–3.3) |
| Officer                               | 1411                  | 91 (6.5)                                      | 1.1 (0.9–1.4) |
| **Military service branch**           |                       |                                               |              |
| Army                                  | 3400                  | 251 (7.4)                                     | Reference    |
| Navy                                  | 2800                  | 109 (3.9)                                     | 0.5 (0.4–0.6) |
| Air Force                             | 2600                  | 143 (5.5)                                     | 0.7 (0.6–0.9) |
| Marine Corps                          | 1200                  | 94 (7.8)                                      | 1.0 (0.8–1.4) |
| **Length of service quartile, years** |                       |                                               |              |
| 0–1.8                                 | 2496                  | 134 (5.4)                                     | Reference    |
| 1.9–4.9                               | 2497                  | 118 (4.7)                                     | 0.9 (0.7–1.1) |
| 5.0–11.2                              | 2499                  | 160 (6.4)                                     | 1.2 (0.9–1.5) |
| >11.2                                 | 2496                  | 185 (7.4)                                     | 1.4 (1.1–1.8) |
| **Military occupational specialty**   |                       |                                               |              |
| Not ground combat                     | 8926                  | 495 (5.6)                                     | Reference    |
| Ground combat                         | 1074                  | 102 (9.5)                                     | 1.8 (1.4–2.2) |

**NOTE.** Samples were tested for all 10,000 subjects. Results are not shown for unknown categories of characteristics with incomplete data. RMSF, Rocky Mountain spotted fever.

* Univariable analysis.
samples were obtained within 7 days after entering the military. Of these 696 subjects, 24 (3.4%) were seropositive (95% CI, 2.1%–4.8%). In univariable logistic regression analysis, only home state RMSF risk was statistically significantly associated with seropositivity (11 [7.6%] of 144 subjects from states with higher RMSF incidences vs. 13 [2.4%] of 538 subjects from states with low RMSF incidence; \( P = .004 \)). In contrast with results in the full study population, antibody prevalence was lower among men (19 [3.4%] of 561 subjects) than among women (5 [3.7%] of 135 subjects) and lower among black individuals (5 [3.4%] of 148 subjects) than among white individuals (18 [4.1%] of 443 subjects). Seropositivity remained least common among Hispanic individuals (1 [1.0%] of 96 subjects). For other variables, sample sizes were too small for reliable estimation.

Antibodies against *A. phagocytophilum* were detected by ELISA in 111 (1.1%) of the 9987 subjects with sufficient serum volume for analysis (95% CI, 0.9%–1.3%). Results were equivocal for an additional 150 subjects. Combining ELISA seropositive and equivocal results, 261 (2.6%) of 9987 subjects (95% CI, 2.3%–2.9%) may have been exposed previously to *A. phagocytophilum*. Univariable logistic regression showed a statistically significant positive association between ELISA seropositivity and college or higher versus high school or less education and showed a statistically significant negative association between ELISA seropositivity and ground combat specialty (table 4). In Western blot testing of the 261 ELISA positive and equivocal serum samples, 11 were positive, giving an *A. phagocytophilum* seroprevalence of 0.11% (95% CI, 0.05%–0.18%). There were no significant associations between Western blot seropositivity and demographic or occupational characteristics. Subjects with positive Western blot results had been in military service for a minimum of 1.9 years when serum samples were obtained. None of the serum samples with antibodies against *A. phagocytophilum* in Western blot testing had ELISA results positive for SFG rickettsiae.

### DISCUSSION

This study is unique in that it shows that SFG rickettsiae exposure is common in a broadly diverse US population. Serological studies involving populations considered to be at elevated risk of tick-borne diseases have reported high proportions with antibodies reactive to *R. rickettsii*, reflecting exposure to *R. rickettsii* or other SFG rickettsiae. In a study of 1999 children from 6 southeastern and south-central states, from whom blood samples had been obtained for any reason at pediatric referral centers, 12% had indirect fluorescence antibody titers to *R. rickettsii* of at least 1:64 [14]. Thirty-two (9.1%) of 352 sixth-graders in an area of Texas with high RMSF incidence had indirect fluorescence antibody titers of at least 1:64; 8 of the seropositive children had experienced fever with rash or headache during the previous year, but none had been hospitalized [15]. High seroconversion rates for *R. rickettsii* were reported among National Guardsmen training at Fort Chaffee, Arkansas, with recent tick attachment and no or mild febrile illnesses [16, 17].

It is unlikely that infection with *R. rickettsii* frequently (or ever) causes mild symptoms [8], and the additional agents responsible for the high seroprevalence of SFG rickettsiae in our study and previous studies are unknown. Of 3 cases of *R. parkeri* rickettsiosis described to date, 2 occurred in service members in the tidewater region of eastern Virginia who developed eschar and febrile illness following tick exposure [6, 7]; the third case was identified in Mississippi [5]. The incidence of *R. parkeri* infection in the United States is unknown, but a recent analysis of 15 serum specimens obtained from US patients with indirect fluorescence antibody seropositivity to *R. rickettsii* found that 4 serum specimens had positive results of an *R. parkeri*–specific Western blot, suggesting that other infections had occurred [18]. Several other SFK rickettsiae endemic to the United States are carried by human-biting ticks and may cause human disease [8]. For example, *R. massiliae*, previously implicated in human illness in Europe and detected…

### Table 3. Final logistic regression model for spotted fever group rickettsiae seropositivity.

| Characteristic                        | OR (95% CI) |
|--------------------------------------|-------------|
| Age quartile, years                  |             |
| 17–22                                | Reference   |
| 23–26                                | 1.0 (0.8–1.4) |
| 27–33                                | 1.2 (0.9–1.5) |
| >=34                                 | 1.4 (1.1–1.8) |
| Sex                                  |             |
| Female                               | Reference   |
| Male                                 | 2.2 (1.6–3.1) |
| Ethnicity                            |             |
| White, non-Hispanic                  | Reference   |
| Black, non-Hispanic                  | 1.4 (1.2–1.8) |
| Hispanic                             | 0.7 (0.5–1.1) |
| Other                                | 0.2 (0.0–1.4) |
| RMSF incidence in home state         |             |
| <US incidence                        | Reference   |
| >=US incidence                       | 1.8 (1.5–2.2) |
| Military service branch              |             |
| Army                                 | Reference   |
| Navy                                 | 0.6 (0.5–0.8) |
| Air Force                            | 0.9 (0.7–1.1) |
| Marine Corps                         | 1.1 (0.8–1.4) |
| Military occupational specialty      |             |
| Not ground combat                    | Reference   |
| Ground combat                        | 1.4 (1.1–1.9) |

**NOTE.** Variables shown remained in the model following backward stepwise elimination from the full model with all variables. Only subjects with data for all variables in the full model were included in this analysis (n = 8352); RMSF, Rocky Mountain spotted fever.
in Africa, recently was isolated from a known R. rickettsii tick vector in Arizona [19].

Independent risk factors for SFG rickettsiae seropositivity in our study included age (likely reflecting cumulative exposure over several years), home state with RMSF incidence at least as high as the US incidence (which may correlate with risk of other tick-borne infections), and ground combat occupational specialties (which may involve greater exposure to tick-infested environments). We observed discrepancies between associations in the entire study population and in the subgroup of subjects whose serum samples were obtained at entry to military service, reflecting service-associated exposures or other factors. In the entire population, in both univariable and multivariable analysis, male subjects were associated with an \( \sim 2 \)-fold higher se-

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**Table 4. Serological results for Anaplasma phagocytophilum.**

| Characteristic                        | No. of samples tested by ELISA | No. (%) of samples with positive or equivocal ELISA results | OR\(^{a}\) (95% CI) | No. (%) of samples with positive Western blot results\(^{b}\) | OR\(^{a}\) (95% CI) |
|---------------------------------------|---------------------------------|------------------------------------------------------------|---------------------|------------------------------------------------------------|---------------------|
| Age quartile, years                   |                                 |                                                            |                     |                                                            |                     |
| 17–22                                 | 2496                            | 57 (2.3)                                                   | Reference           | 1 (0.0)                                                    | Reference           |
| 23–26                                 | 2498                            | 58 (2.3)                                                   | 1.0 (0.7–1.5)       | 3 (0.1)                                                    | 3.0 (0.3–28.9)      |
| 27–33                                 | 2497                            | 78 (3.1)                                                   | 1.4 (1.0–2.0)       | 3 (0.1)                                                    | 3.0 (0.3–28.9)      |
| >34                                   | 2496                            | 68 (2.7)                                                   | 1.2 (0.8–1.7)       | 4 (0.2)                                                    | 4.0 (0.4–35.9)      |
| Sex                                   |                                 |                                                            |                     |                                                            |                     |
| Female                                | 1571                            | 47 (3.0)                                                   | Reference           | 3 (0.2)                                                    | Reference           |
| Male                                  | 8416                            | 214 (2.5)                                                  | 0.8 (0.6–1.2)       | 8 (0.1)                                                    | 0.5 (0.1–1.9)       |
| Ethnicity                             |                                 |                                                            |                     |                                                            |                     |
| White, non-Hispanic                   | 6772                            | 168 (2.5)                                                  | Reference           | 5 (0.1)                                                    | Reference           |
| Black, non-Hispanic                   | 1965                            | 65 (3.3)                                                   | 1.3 (1.0–1.8)       | 4 (0.2)                                                    | 2.7 (0.7–10.2)      |
| Hispanic                              | 1105                            | 27 (2.4)                                                   | 1.0 (0.7–1.5)       | 2 (0.2)                                                    | 2.5 (0.5–12.7)      |
| Other                                 | 125                             | 1 (0.8)                                                    | 0.3 (0.0–2.3)       | 0 (0.0)                                                    | NA                  |
| Education                             |                                 |                                                            |                     |                                                            |                     |
| High school or lower                  | 6309                            | 144 (2.3)                                                  | Reference           | 6 (0.1)                                                    | Reference           |
| College or higher                     | 3302                            | 107 (3.2)                                                  | 1.4 (1.1–1.8)       | 5 (0.2)                                                    | 1.6 (0.5–5.2)       |
| HGA incidence in home state           |                                 |                                                            |                     |                                                            |                     |
| <US incidence                         | 6529                            | 171 (2.6)                                                  | Reference           | 10 (0.2)                                                   | NA                  |
| >US incidence                         | 981                             | 26 (2.7)                                                   | 1.0 (0.7–1.5)       | 0 (0.0)                                                    |                     |
| Military rank                         |                                 |                                                            |                     |                                                            |                     |
| Enlisted                              | 8458                            | 216 (2.6)                                                  | Reference           | 9 (0.1)                                                    | Reference           |
| Warrant officer                       | 118                             | 3 (2.5)                                                    | 1.0 (0.3–3.2)       | 0 (0.0)                                                    | NA                  |
| Officer                               | 1411                            | 42 (3.0)                                                   | 1.2 (0.8–1.6)       | 2 (0.1)                                                    | 1.3 (0.3–6.2)       |
| Military service branch               |                                 |                                                            |                     |                                                            |                     |
| Army                                  | 3390                            | 89 (2.6)                                                   | Reference           | 4 (0.1)                                                    | Reference           |
| Navy                                  | 2800                            | 64 (2.3)                                                   | 0.9 (0.6–1.2)       | 3 (0.1)                                                    | 0.9 (0.2–4.1)       |
| Air Force                             | 2597                            | 88 (3.4)                                                   | 1.3 (1.0–1.8)       | 3 (0.1)                                                    | 1.0 (0.2–4.4)       |
| Marine Corps                          | 1200                            | 20 (1.7)                                                   | 0.6 (0.4–1.0)       | 1 (0.1)                                                    | 0.7 (0.1–6.3)       |
| Length of service quartile, years     |                                 |                                                            |                     |                                                            |                     |
| 0–1.8                                 | 2493                            | 63 (2.5)                                                   | Reference           | 0 (0.0)                                                    | NA                  |
| 1.9–4.9                               | 2495                            | 66 (2.7)                                                   | 1.0 (0.7–1.5)       | 4 (0.2)                                                    |                     |
| 5.0–11.2                              | 2496                            | 66 (2.7)                                                   | 1.0 (0.7–1.5)       | 3 (0.1)                                                    |                     |
| >11.2                                 | 2492                            | 66 (2.7)                                                   | 1.0 (0.7–1.5)       | 4 (0.2)                                                    |                     |
| Military occupational specialty       |                                 |                                                            |                     |                                                            |                     |
| Not ground combat                     | 8916                            | 244 (2.7)                                                  | Reference           | 11 (0.1)                                                   | NA                  |
| Ground combat                         | 1071                            | 17 (1.6)                                                   | 0.6 (0.3–0.9)       | 0 (0.0)                                                    |                     |

**NOTE.** Samples were tested for 9987 subjects with sufficient serum volume for analysis. Results are not shown for unknown categories of characteristics with incomplete data. HGA, human granulocytic anaplasmosis; NA, not applicable.

\(^{a}\) Univariable analysis.

\(^{b}\) Only serum samples with positive and equivocal ELISA results were tested by Western blot.
or mild infection with SFG rickettsiae and A. phagocytophilum is unknown. In studies involving patients with HGA, ~50% became seronegative by 1 year [26, 27] and 92% by 3.5 years [28] after onset of illness. Third, serological assays for R. rickettsii cannot distinguish among the multiple SFG rickettsiae that could exhibit cross-reactivity (although more-specific Western blot analysis confirmed the results for a high proportion of ELISA-positive specimens tested, suggesting that cross-reactivity with non-SFG rickettsiae was uncommon). Fourth, subject deployment histories were unavailable. Exposure in areas with a higher risk of rickettsioses (e.g., Eastern Europe, Africa, and Southeast Asia) could have contributed to the high prevalence of SFG rickettsiae seropositivity.

Despite these limitations, our study suggests that exposure to SFG rickettsiae occurs fairly commonly across a broad geographic range in the United States, whereas the risk of A. phagocytophilum infection is likely to be confined to certain groups and is very low in the general population. The high prevalence of SFG rickettsiae seropositivity in our population supports calls for the development of more-specific serological assays that are capable of distinguishing SFG rickettsiae species and more frequent use of real-time PCR and multilocus sequence typing and cell culture [8, 29]. Incorporation of these tools into epidemiological studies and clinical practice could improve surveillance for RMSF by reducing false-positive reports and could better define the clinical spectrum of SFG rickettsial infections.

Acknowledgments
We thank Dr. Gregory A. Dasch, for purified Rickettsia rickettsii antigen, and Dr. Yasuko Rikihisa, for the Anaplasma phagocytophilum rP44 antigen.

Financial support. US Naval Medical Research Center and Department of Defense Global Emerging Infections Surveillance & Response System.

Potential conflicts of interest. All authors: no conflicts.

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