Seroepidemiologic Survey for *Coxiella burnetii* Among US Military Personnel Deployed to Southwest and Central Asia in 2005

Joseph Royal,* Mark S. Riddle, Emad Mohareb, Marshall R. Monteville, Chad K. Porter, and Dennis J. Faix
Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland;
Naval Medical Research Center, Silver Spring, Maryland; US Naval Medical Research Unit, No. 3, Cairo, Egypt;
Naval Health Research Center, San Diego, California

Abstract. We used a seroepidemiologic study to estimate Q fever (*Coxiella burnetii*) seroprevalence, seroincidence, and risk factors for seroconversion in two deployed military populations in 2005. The first study group resided in an area with a known Q fever outbreak history (Al Asad, Iraq). Of this population, 7.2% seroconverted for an incidence rate of 10.6 seroconversions per 1,000 person-months. The second population included personnel transiting through Qatar on mid-deployment leave from southwest/central Asia. In this group, we found 2.1% prevalence with 0.92 seroconversions per 1,000 person-months. However, no significant risk factors for Q fever seroconversion were found in either population.

INTRODUCTION

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a Gram-negative intracellular pathogen capable of prolonged survival under harsh environmental conditions.¹ Most human cases are thought to result from inhalation of windborne dust contaminated with *C. burnetii* from reproductive tissues of parturient domestic livestock, primarily sheep, goats, and cattle.^{2,3} Disease has occasionally been associated with other routes of exposure, including consumption of unpasteurized milk and tick bites.¹

Multiple case reports and case series have documented the diagnosis and treatment of acute and possible chronic Q fever in recent years in military personnel located in or returning from Iraq and Afghanistan.^{4–9} As a result, the Armed Forces Infectious Disease Society published a set of practice guidelines for the diagnosis and management of Q fever in 2012.¹⁰

In the summer of 2005, a US Marine Corps platoon experienced an outbreak of respiratory disease while deployed to Al Asad in western Iraq.¹¹ The outbreak affected 22 of 38 (58%) Marines in the platoon. After detecting antibodies against *C. burnetii* in 9 personnel, follow-up questionnaires were completed by 132 of 136 personnel in the affected platoon's company to determine the extent of the Q fever outbreak and identify possible risk factors for infection. Based on epidemiologic analysis, investigators identified possible risk factors as exposure to tick bites, camels, and births of sheep and dogs.

Data on the background incidence of infection, seroprevalence, and risk factors of Q fever in military personnel are limited. The objectives of this study were to estimate the seroprevalence and seroincidence of *C. burnetii* infection in US military personnel deployed to Q fever-endemic areas and identify potential risk factors for infection. Our two study populations were the Marines deployed to Iraq as described above and service members transiting through Doha, Qatar, on mid-deployment leave from southwest and central Asia.

METHODS

Study population. *Al Asad 2005.* As previously reported,¹¹ 132 post-deployment serum samples were obtained from the affected platoon's company through the Department of Defense Serum Repository (DoDSR).¹² Additionally, post-deployment DoDSR samples were also obtained from another reserve unit of 172 Marines operating in the same region that was not involved in the outbreak to assess the extent of the outbreak. Samples were tested for antibodies to *C. burnetii*, and for positive samples, corresponding pre-deployment serum samples were also obtained from the DoDSR and tested. Investigators distributed questionnaires in Iraq to the outbreak group as described previously.¹¹ Surveys were subsequently mailed to the non-outbreak group after they returned to the United States; however, no completed surveys were received from this group. The survey gathered basic demographic information (age, sex, unit, and military rank) and solicited information on self-reported history of febrile illness, exposure to livestock (cattle, sheep, goats, and camels) or animal birthing (cattle, sheep, goats, camels, dogs, and cats), consumption of unpasteurized milk, and tick bites. History of febrile illness was defined as any illness accompanied by fever during the current deployment. Exposure to animals and exposure to animal birth were defined as subjects reporting being exposed to (within 500 m) or seeing any of the listed animals or animal births while deployed. The Al Asad dataset was collected as part of an outbreak investigation and did not require Institutional Review Board (IRB) approval. Informed consent was obtained for all data collected.

Qatar 2005. To assess background rates of *C. burnetii* infection in a deployed population, we used mid-deployment serum samples collected by the Naval Medical Research Unit, No. 3 (NAMRU-3) and pre-deployment DoDSR serum samples linked to questionnaire response data from an additional group of deployed military personnel. This convenience sample consisted of active duty military personnel deployed to various locations in southwest and central Asia identified during their mid-deployment Rest and Recuperation Program (R&R) stay in Doha, Qatar, from July of 2005 to June of 2006. A *priori* power analysis was performed to determine optimal sample size, and subjects were recruited during mandatory in-briefings conducted on their arrival at the study site until 800 subjects were enrolled. Data on demographics, deployment

* Address correspondence to Joseph Royal, Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20877. E-mail: joseph.m.royal.mil@mail.mil

location, time in theater, history of febrile illness, and exposure to arthropod bites were obtained from self-reported questionnaire data collected by the NAMRU-3 Military Infectious Disease and Operational Health Surveillance Network. Identification numbers were used to link survey response data to serologic samples. The Qatar dataset was collected as part of a study protocol (NAMRU3.2005.0009) approved by the NAMRU-3 IRB in compliance with all applicable federal regulations governing the protection of human subjects.

Laboratory testing. Q fever serology was performed using a commercial phase II immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA; PanBio, Brisbane, Australia). An index value (Panbio units) was calculated, and results were characterized as negative (< 9), positive (> 11), or equivocal (9–11) based on manufacturer-established cutoffs. All sero-positive samples were serologically confirmed by double testing using the same kit. For the purposes of this study, equivocal results were counted as negative. For both sample populations, seroconversion was defined as a positive mid- or post-deployment test for the Qatar and Al Asad populations, respectively, coupled with negative pre-deployment results.

Contacting subjects to inform them of test results was not allowed under the IRB approval for the Qatar study, and subjects gave informed consent to this stipulation on enrolling in the study. For the Al Asad outbreak and control subjects, all laboratory testing results were provided to the corresponding medical providers for inclusion in medical records and patient follow-up as appropriate.

Statistical analysis. We analyzed continuous variables using parametric (Student *t* test) or non-parametric (Mann–Whitney *U* or Kruskal–Wallis test) methods and categorical variables using χ^2 or Fisher exact tests as appropriate. We used a logistic regression model to estimate adjusted odds ratios (ORs) for potential Q fever risk factors in the Al Asad population, and we used exposure to dog birth, history of tick bites, and

age as predictors in the model. We calculated point estimates and 95% confidence intervals (95% CIs) for incidence rates using data on seroconversion and person-time in theater based on a Poisson distribution. Analyses were conducted using Stata version 10 (Stata Corp., College Station, TX), and statistical significance was set as a two-tailed $P \leq 0.05$.

RESULTS

Al Asad 2005. A total of 304 subjects were included in this study population, and 281 subjects had post-deployment serum available for testing. Of these subjects, 26 (9.3%) subjects tested positive for Q fever phase II IgG (Figure 1). There were 279 subjects eligible for seroconversion (excluding 2 subjects missing pre-deployment serum), of which 20 (7.2%) subjects seroconverted; 4 (1.4%) subjects were seropositive cases identified before deployment. Surveys were completed for only 109 of 281 (39%) subjects receiving post-deployment serologic testing, and 17 (16%) subjects were seroconverters (Table 1). Of these subjects, 14 subjects had previously been identified as clinical cases during the initial outbreak investigation. There were 3 (1.8%) subjects with seroconversion identified in 170 subjects from the non-outbreak group with pre-deployment serum available for testing. Subjects with serologic data accumulated approximately 1,882 person-months of deployment time, and the incidence of seroconversion was 10.6 per 1,000 person-months (95% CI = 6.1–15.4).

Seroconverters had a median age of 24 years (range = 21–34 years), and most (88%) were of junior enlisted ranks. Non-seroconverters had a median age of 23 years (range = 19–41 years), which did not differ significantly between the two groups ($P = 0.25$); 16 (94%) of 17 seroconverters reported a history of febrile illness during the deployment compared with 26% (24/91) of non-seroconverters ($P < 0.001$). In the logistic regression analysis, exposure to dog births

FIGURE 1. Al Asad outbreak flow chart showing results of pre- and post-deployment serology and number within each group that completed surveys.

TABLE 1
Al Asad 2005 survey results by seroconversion status

Self-reported variables	Seroconverters (N = 17)	Non-seroconverters (N = 92)	OR	95% CI low	95% CI high	P value
Demographics						
Age group (years; %)						
< 25	9/17 (53)	57/86 (66)	0.57	0.18	1.9	0.44
25–29	5/17 (29)	20/86 (23)	1.4	0.33	4.8	0.79
≥ 30	3/17 (18)	9/86 (10)	1.8	0.28	8.6	0.62
Rank* (%)						
Junior enlisted	15/17 (88)	71/91 (78)	2.1	0.43	20	0.52
Senior enlisted	2/17 (12)	16/91 (18)	0.63	0.064	3.2	0.73
Officer	0/17 (0)	4/91 (4.4)	NE	NE	NE	NE
Risk factors						
Animal exposure (%)						
Cattle	11/16 (69)	81/92 (88)	0.30	0.077	1.3	0.12
Sheep	14/17 (82)	89/92 (97)	0.16	0.020	1.3	0.095
Goats	13/16 (81)	88/92 (96)	0.20	0.030	1.5	0.13
Camels	5/16 (31)	30/90 (33)	0.91	0.23	3.2	1.0
Birth exposure (%)						
Cattle birth	2/16 (13)	19/72 (21)	0.40	0.041	2.0	0.40
Sheep birth	6/17 (35)	24/92 (26)	1.5	0.42	5.2	0.61
Goat birth	4/16 (25)	24/92 (26)	0.94	0.20	3.5	1.0
Camel birth	1/16 (6.3)	7/91 (7.7)	0.80	0.017	7.0	1.0
Dog birth	8/17 (47)	26/92 (28)	2.2	0.67	7.4	0.21
Cat birth	4/16 (25)	24/92 (26)	0.94	0.20	3.5	1.0
Other risk factors (%)						
Unpasteurized milk	0/17 (0)	2/91 (2.2)	NE	NE	NE	NE
Tick bites	3/17 (18)	4/90 (4.4)	4.5	0.60	30	0.16

Self-reported demographics, history of febrile illness during deployment, and potential risk factors for Q fever exposure. Survey responses of no or uncertain were categorized as no. All ORs are unadjusted odds ratios. NE = not evaluable.

* Junior enlisted category includes pay grades E1–E4; senior enlisted category includes pay grades E5–E9.

(adjusted OR = 2.5, 95% CI = 0.84–7.5; $P = 0.099$) and history of tick bites (adjusted OR = 5.1, 95% CI = 1.0–27; $P = 0.056$) seemed to be associated with increased risk of infection (with exposure to tick bites approaching statistical significance).

Qatar 2005. A total of 800 subjects were enrolled in the 2005 seroepidemiology study in Qatar (Figure 2). The median

age of the participants was 26 years (range = 18–60 years), and 87% were male. Subjects were most commonly US Army personnel (76%) deployed to Iraq (70%); one-half were of junior enlisted rank.

Of 800 participants, mid-deployment serum samples were available for 584 (73%) participants for Q fever antibody testing; 12 (2.1%) samples were positive for Q fever phase II IgG.

FIGURE 2. Qatar seroepidemiology study flow chart showing results of pre- and mid-deployment serology results.

One subject was missing pre-deployment serum. The remaining 583 subjects accounted for 3,250 person-months of deployment time, with a median duration of 170 days (interquartile range = 114–226 days). Three (0.51%) samples showed evidence of seroconversion, resulting in an incidence rate of 0.92 seroconversions per 1,000 person-months (95% CI = 0.30–2.9). All three seroconverters were men deployed to Iraq. Two subjects reported a history of fever with chills, sweats, nausea, extreme fatigue, headache, and myalgia during the deployment. Neither subject reported insect or tick bites in 14 days preceding their most recent febrile episodes. No statistically significant risk factors for seroconversion were identified. There were eight (1.4%) seropositive cases identified before deployment. The median age of these individuals was 27 years (range = 22–47 years).

DISCUSSION

Pre-deployment seroprevalence was approximated under the assumptions that no seroreversions occurred and that the two seropositive post-deployment Al Asad samples and one seropositive mid-deployment Qatar sample with missing pre-deployment sample represented seroconversions and not prevalent cases pre-deployment. On this basis, pre-deployment crude seroprevalence in both study populations was 1.4%. By comparison, the age-specific seroprevalence in a 2003–2004 US sample was approximately 1.4% and 2.4% among individuals ages 20–29 and 30–39 years, respectively.¹³ This finding suggests that the pre-deployment seroprevalence in our population was comparable with a same-age civilian population.

We found a post-deployment seroprevalence in the Al Asad outbreak group of 9.3%. However, much higher seroprevalence has been observed in other locations and groups: 32% in blood donors in Turkey,¹⁴ 23% in blood donors in Spain,¹⁵ and 22% in US veterinarians.¹⁶ This finding likely can be attributed to differences in risk by region, exposure time in endemic areas, population demographics, occupational exposures, and diagnostic methodologies.

A recent seroepidemiologic study reported an overall seroincidence of 10% (88 of 909) in US military personnel hospitalized during their deployment to Iraq from April of 2003 to December of 2004 with a diagnosis suggestive of possible Q fever.¹⁷ The overall seroincidence in the Al Asad group was lower (7.2%) but not significantly different ($P = 0.20$), whereas the seroincidence of the Qatar group (0.51%) was significantly lower ($P < 0.0001$). However, these populations may not be comparable given the differences in selection criteria, demographics, occupational specialties, dates of deployment, and travel locations. Additionally, Anderson and others¹⁷ used a different laboratory testing methodology (i.e., indirect immunofluorescent antibody assay) to detect phases I and II IgG and IgM antibodies.

The higher seroincidence rate in the Al Asad population (10.6 of 1,000) compared with the Qatar population (0.92 of 1,000) was not surprising given that the Al Asad population included subjects previously identified during an outbreak investigation. As part of their assigned mission, the Marines in Al Asad traveled relatively long distances searching for insurgents and weapons caches, establishing vehicle checkpoints, and securing bases of operation. As part of these missions, personnel sometimes passed through or took shelter in areas where livestock were present, resulting in potential exposure to *C. burnetii*.

Furthermore, large amounts of airborne particulate matter and dust are common in such an environment because of dust storms or entering and exiting helicopters, providing a potential route for exposure to infectious material. Although important in the epidemiology of Q fever, some of these risk factors were not assessed or easily quantifiable and therefore, could not be evaluated.

Although acute Q fever is thought to be asymptomatic in approximately one-half of cases,¹⁸ our study suggests that lower rates of asymptomatic infection occurred in the Al Asad outbreak. Of 17 seroconverters, it is unlikely that more than one asymptomatic seroconversion occurred given that 14 seroconverters were previously identified as clinical cases,¹¹ and 2 of the non-clinical cases reported experiencing a febrile illness on the questionnaire.

In the Al Asad study, history of tick bites and exposure to dog births seemed to increase risk for seroconversion. However, the study was underpowered to identify Q fever-specific risk factors. Ticks are known to carry *C. burnetii* and may be a source of sporadic Q fever cases in humans.² It is also possible that rural areas where livestock are prevalent coincide with areas of denser tick populations. Individuals spending more time on patrols in rural areas may have concurrently increased risks of exposure to Q fever and tick bites. Dogs can be infected with *C. burnetii* and have been suspected as a source of Q fever in humans.^{19,20} The Q fever seroprevalence in feral dogs in Iraq was 5.5% from 2007 to 2008.²¹ It is, therefore, conceivable that exposure to dogs could have contributed to the outbreak in Al Asad.

Limitations of this study include non-response bias in the Al Asad Q fever outbreak investigation, where higher seroconversion rates were identified in survey responders (17 of 109, 16%) compared with non-responders (3 of 170, 1.8%; $P < 0.0001$). Furthermore, as previously discussed by Faix and others,¹¹ a retrospective survey of this type could also be susceptible to recall bias, which could lead to overestimation of some risk factor estimates.¹¹ For this reason, incidence rates and risk factor conclusions from the outbreak group may not extrapolate well to the general deployed military population. The much lower rate in the non-outbreak group of the Al Asad study suggests that the Q fever outbreak did not extend much beyond those individuals identified in the initial investigation.

For all subjects in the outbreak investigation, detailed demographic data were not available for comparison or did not vary (e.g., all subjects were male Marines). The Qatar study was designed to evaluate diseases other than Q fever. Consequently, questionnaires did not address several Q fever-specific risk factors.

Misclassification caused by testing methodology may have affected our results. Published sensitivities of the ELISA used in this study range from 71% to 90%, and specificity estimates range from 96% to 99.7%.^{22–24} Because the test used in this study is not perfectly sensitive or specific, it is possible that our datasets may contain false positives and false negatives that could substantially alter the estimated seroprevalence. Furthermore, recent infections may have been missed because of a lack of detectable phase II IgG antibody. Future studies may incorporate additional testing of negative and equivocal results for phase II IgM. Another limitation is that the ELISA assay used in this study has seen only limited validation.^{22–24} Future studies of this kind might use other test methods,

such as indirect immunofluorescence, which is generally considered the reference technique for Q fever screening.¹

CONCLUSION

Limited data are available on Q fever epidemiology in military-relevant regions. We also know very little about incidence of Q fever in military populations, making it difficult to efficiently identify cases and assess risk, burden of disease, and need for interventions. This study provides estimates of *C. burnetii* infection in deployed military personnel, which may be used to assess medical risks in deployment operations and guide future research in diseases of military importance. Furthermore, by identifying previously undetected cases of infection, we now have a clearer picture of the extent of a Q fever outbreak among a unit of Marines deployed to Iraq. This information will better inform policies and resource allocation for detection and prevention of Q fever in deployed military personnel in future operations.

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Authors' addresses: Joseph Royal, Uniformed Services University of the Health Sciences, Bethesda, MD, E-mail: joseph.m.royal.mil@mail.mil. Mark S. Riddle, Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD, E-mail: Mark.Riddle@med.navy.mil. Emad Mohareb and Marshall R. Monteville, US Naval Medical Research Unit, No. 3, Cairo, Egypt, E-mails: Emad.Mohareb.eg@med.navy.mil and Marshall.Monteville@med.navy.mil. Chad K. Porter, Naval Medical Research Center, Silver Spring, MD, E-mail: Chad.Porter@med.navy.mil. Dennis J. Faix, Naval Health Research Center, San Diego, CA, E-mail: Dennis.Faix@med.navy.mil.

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