

An Ongoing Series

Arthropod-Borne and Zoonotic Infections Among Military Personnel in Georgia

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ABSTRACT

Military personnel are at an increased risk for exposure to arthropod-borne and zoonotic pathogens. The prevalence of these pathogens has not been adequately described in the country of Georgia. As the Georgian military moves toward an increased level of capability and the adoption of European Union and North Atlantic Treaty Organization standards, international field exercises will become more frequent and will likely involve an increasing number of international partners. This study was undertaken with the goal of defining the arthropod-borne and zoonotic pathogen threat in Georgia so force health protection planning can proceed in a rational and data-driven manner. To estimate disease burden, blood was taken from 1,000 Georgian military recruits between October 2014 and February 2016 and screened for previous exposure to a set of bacterial and viral pathogens using an antibody-based, serologic procedure. The highest rate of exposure was to *Salmonella enterica* serovar Typhi, and the lowest rate of exposure was to *Coxiella burnetii* (the causative agent of Q fever). These data provide insight into the prevalence of arthropod-borne infections in Georgia, fill a critical knowledge gap, will help guide future surveillance efforts, and will inform force health protection planning.

KEYWORDS: *arthropod-borne infection; zoonotic infection; zoonoses; epidemiology; Georgian military*

Introduction

The prevalence of human exposure to arthropod-borne and zoonotic infectious agents in the country of Georgia has not been adequately described. A greater understanding of the temporal and spatial patterns of infection will provide substantial insight into the epidemiology and possible control of

these infections at the population level. Increased knowledge regarding the burden and distribution of pathogens in the active-duty military population will provide valuable information for the development and implementation of force health protection plans and aid in the formation of the situational awareness of military decision makers.¹ In addition, military personnel represent ideal surrogates for estimating the prevalence of zoonotic and arthropod-borne infections in the general population, because they are typically young, active, and healthy. Such a population is capable of mounting a robust immune response to pathogen exposure and, therefore, is likely to have a reasonably intact immunologic memory facilitating serologic analysis.² Also, because recruits are drawn from throughout the country, the military recruit population has a diverse geographic and demographic profile that is broadly representative of the population as a whole.

Thus, we conducted a pathogen-specific, antibody seroprevalence study among military recruits as they underwent pre-contract medical and health assessment at the Central Military Hospital in Gori, the primary military hospital of the Georgian armed forces. Study participants were serologically tested for prior exposure to a select group of arthropod-borne and zoonotic infections, including Crimean-Congo hemorrhagic fever, hemorrhagic fever with renal syndrome, tick-borne encephalitis, West Nile virus (WNV), brucellosis, leptospirosis, tularemia, anthrax, rickettsia, Q fever, and *Salmonella enterica* serovar Typhi.³⁻⁵

To our knowledge, a military-recruit specific seroprevalence study has not been conducted of most of these diseases in Georgia. In this study, previous infection was defined as prior exposure to the infectious agent and a detectable pathogen-specific

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antibody response was used as an indicator of previous infection. The primary objective of this study was to determine serologic evidence of exposure to 14 arthropod-borne and zoonotic infections prior to military recruitment and to determine the epidemiological risk factors associated with pathogen exposure.

Materials and Methods

Study Site

The Military Hospital of the Ministry of Defense of Georgia was chosen as the study site. This hospital is the primary treatment center for the Georgian military and where all recruits intending to serve in the Georgian Army undergo their pre-recruitment medical assessment. Individuals were invited to participate in the study at the time of this assessment. Enrolled volunteers were required to be at least 18 years of age at the time of enrollment and to agree to participate in this study by signing an informed consent document.

Data Collection

A questionnaire about previous illnesses and exposures to risk factors was completed for each volunteer and 10mL of blood was collected for study purposes. All blood samples were collected in anticoagulant-free tubes and stored at -20°C until processing. Commercial enzyme-linked immunosorbent assays (ELISAs) and immunofluorescence assays were used to evaluate each sample for antibody response to the pathogens under study.^{6,7}

Pathogens Evaluated

Samples were tested serologically for antibody response to the following pathogens: *Bacillus anthracis*, *Brucella* spp, Crimean Congo hemorrhagic fever virus, *Coxiella burnetii*, *Francisella tularensis*, hantavirus, *Rickettsia* spp, tick-borne encephalitis virus, *Bartonella* spp, *Borrelia* spp, *Ehrlichia* spp, *Leptospira* spp, *S typhi*, and WNV. *C burnetii* and *B burgdorferi* were tested for at the Military Hospital; the rest of the assays were performed by technologists at the National Center for Disease Control and Public Health of Georgia.

Statistical Analyses

Data were entered into an Epi Info™ database (Centers for Disease Control and Prevention, <https://www.cdc.gov>). Epi Info, version 7, and SPSS, version 24 (IBM, <https://www.ibm.com>), were used for data analysis. Odds ratios along with 95% confidence intervals were calculated to estimate associations between risk factors and study outcomes by univariate logistic regression analysis.⁸

Results

Enrollment Demographics

From October 2014 to February 2016, a total of 1,000 recruits were enrolled in this study. Most of the enrollees (98%) were male; the median age was 30 years (range, 18–52 years).⁹ Vector exposure was widespread, with 89% of the enrollees self-reporting being bitten by mosquitoes, 35% reporting rodents in their houses, 8% reporting being bitten by fleas, 4% reporting being bitten by ticks, and 1% reporting exposure to lice. The largest number of enrollees were from the Shida Kartli region in central Georgia.¹⁰ The lowest number of enrollees came from the Racha Lechkhumi and Kvemo Svaneti region of northwestern Georgia, and the rest of the enrollees

were from various regions throughout the country, with 17% originating in Imereti in the west-central portion of the country (Figure 1; Table 1).

FIGURE 1 Map of Georgia showing an outline of each administrative division (region). (Adapted from d-maps.com.¹⁰)



TABLE 1 Demographic Characteristics of Volunteers in This Seroprevalence Study of Military Personnel, October 2014–February 2016

Characteristic	No. (%) ^a
Age, mean (SD), years	31.2 (7)
Median	30
Sex	
Male	978 (98)
Female	18 (1.8)
Missing data	1 (0.1)
Regional distribution	
Tbilisi	226 (23)
Imereti	168 (17)
Kvemo Kartli	109 (11)
Shida Kartli	227 (23)
Kakheti	54 (5)
Samegrelo-Zemo Svaneti	97 (10)
Ajara	44 (4)
Other	72 (7)
Ethnicity	
Georgian	983 (99)
Other	14 (1)
Education	
Middle school	22 (3)
High school	640 (64)
>High school	331 (33)

SD, standard deviation.

^aUnless otherwise indicated.

ELISA-Based Seroprevalence

Table 2 lists seroprevalence rates of recorded in this study. The highest rate of seroprevalence was for *S Typhi* (23%).

The lowest rates of seroprevalence were for *C burnetii* and WNV (0.6% and 0.8%, respectively). Approximately 1% of the participants were seropositive for *B burgdorferi*, the causative agent of Lyme disease, and about 4% were seropositive for either the spotted fever or the typhus group of the *Rickettsia* spp.³ There was a seropositivity rate of 2.8% for *Leptospira* spp and 5.7% for *Bartonella* spp; these organisms are re-emerging bacterial pathogens that can result in severe life-threatening infections if left untreated.^{3,11} Of the samples that were positive for agents that have been characterized as

TABLE 2 Laboratory Testing Results of Seroprevalence Study in Military Personnel, October 2014–February 2016

Assay	Seropositive patients, No. (%)
<i>Brucella</i> spp IgG ELISA	51 (5)
<i>Leptospira</i> spp IgG ELISA	28 (2.8)
<i>Coxiella burnetii</i> , IgG ELISA	6 (0.6)
<i>Salmonella enterica</i> serovar Typhi IgG ELISA	233 (23)
CCHFV IgG ELISA	10 (1)
Hantavirus IgM ELISA	59 (5.9)
WNV IgG ELISA	8 (0.8)
<i>Francisella tularensis</i> IgG ELISA	61 (6.1)
<i>Bacillus anthracis</i> IgG ELISA	32 (3.2)
<i>Rickettsia</i> Typhus group IgG ELISA	19 (1.9)
<i>Rickettsia</i> Spotted Fever group IgG ELISA	22 (2.2)
<i>Borrelia burgdorferi</i> IgG ELISA	13 (1.3)
<i>Bartonella henselae</i> /B <i>quintana</i> IgG	57 (5.7)

CCHFV, Crimean-Congo hemorrhagic fever; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IgM, immunoglobulin M; WNV, West Nile virus.

especially dangerous pathogens, 3.2% were seropositive for *Bacillus anthracis*, 5% were positive for *Brucella* spp, and about 6% were positive for either *F tularensis* or hantavirus.

The largest number of total seropositive patients came from the Shida-Kartli region in east-central Georgia and the lowest number of total seropositive patients came from the Racha-Lechkhumi and Kvemo Svaneti region in northern Georgia (Figure 1; Table 3). Significantly, *S* Typhi and *Brucella* spp. were the only two bacterial agents and hantavirus was the only viral agent that gave positive serologic results in sera collected from patients originating from every region of Georgia, which potentially indicates widespread distribution. It was noted that rural residence alone was not related to the rate of seropositivity (in this study, sera positive for all of the agents under study were only derived from patients originating from Tbilisi, the capital city of Georgia). Logistic regression analysis of risk factors did not reveal statistically significant differences in seropositivity for exposure to any agent under study between groups with or without known risk factors. Furthermore, none of the participants had a medical history that was significant for signs or symptoms associated with any of the pathogens evaluated.

Discussion

Interestingly, the highest seroprevalence was detected for *S* Typhi. Although this result may indicate a high degree of prevalence of this organism in Georgia, it is also possible that it stems from antibody cross-reactivity.¹² Cross-reactivity occurs intraspecifically between various *Salmonella* spp serovars and interspecifically between *Salmonella* spp and various non-*Salmonella* pathogens, such as members of the genus *Brucella* or other members of the Enterobacteriaceae.¹³ Because neither culture nor molecular diagnostics were used in this study, additional research will be needed to resolve this discrepancy. However, it is interesting to note that although anecdotal evidence suggests that international visitors to Georgia often have diarrheal disease soon after arrival, typhoid fever has not been recently detected in Georgia by active or passive surveillance.

Because *Salmonella* spp have not been extensively characterized in Georgia, it is possible that unique or undescribed

strains of *Salmonella* are present in Georgia and that these are responsible for the high degree of prevalence noted in this study and many of the reported cases of gastrointestinal illness. This is an especially attractive hypothesis given that *Salmonella* exposure was detected in sera of patients from every region of the country and given that most patients originated from the capital of Tbilisi (where most travelers tend to stay during their visit). Furthermore, it can be hypothesized that native Georgians have developed an immunity to many of the endemic *Salmonella* strains via repeated exposure, whereas visitors, lacking a history of repeated exposure, are susceptible to illness.¹⁴ If true, this hypothesis would resolve the discrepancy between the high level of seroprevalence among Georgian military recruits and the general absence of reported symptoms.

Interestingly, similar seroprevalence was detected between common and uncommon pathogens. For example, the seroprevalence for *Brucella* spp, hantavirus, *F tularensis*, and *Bartonella* spp were 5%, 5.9%, 6%, and 5.7%, respectively. Among these pathogens, *Brucella* spp are endemic to Georgia and are a common cause of human disease (100–150 cases per year), *F tularensis* is endemic to Georgia but disease cases are rare (1–2/year), hantavirus infection is rarely described, and *Bartonella* is an uncommon but emerging pathogen.^{15–17}

The seroprevalence of *F tularensis* was low compared with that of neighboring countries. For example, the seroprevalence of *F tularensis* in Azerbaijan was greater than 15%.¹⁶ However, this may be due to sampling error, and although it is reflective of the military-age population in Georgia, it may not reflect the population as a whole.

Oral communication with clinicians has revealed that Lyme disease is often encountered in routine clinical practice in Georgia. However, presently, there is very little published information about prevalence or geographic distribution of this disease in the region. Recent reports have listed Lyme borreliosis among the endemic diseases in Abkhazia, a breakaway region of Georgia, and it is well known that Lyme disease is common in several neighboring countries, including Turkey and Russia.¹⁷ In addition, the vector of Lyme disease, *Ixodes ricinus*, has been detected at 67 locations throughout Georgia.¹⁸ As a whole, these factors indicate that Lyme borreliosis is a potentially important and underdiagnosed disease in this country. However, only 1% of the patients in this study were seropositive for *Borrelia* spp, and this factor was not positively associated with regions bordering endemic countries. Additional research will be required to fully characterize the geographic spread of this disease and to identify regional reservoirs and hot spots.

Throughout this study, the clinical correlates of infection did not always match serologic results. This may suggest that seropositivity in these cases was primarily an indicator of sub-clinical exposure rather than of previous clinically manifested illness. There were 74 participants who were seropositive for more than one type of infectious agent and few were seropositive for up to seven pathogens simultaneously. These findings suggest that a high rate of pathogen exposure is occurring in Georgia without the development of clinical disease in the native-born population. A greater understanding of the nature of these exposures and how they relate to clinical illness and host immune status will be essential for the advancement of force health protection and public health efforts.

TABLE 3 Distribution of Seropositive Cases Across the Regions of Georgia

Region	No. of Cases	Pathogen Immunoassay										
		Brucella spp IgG	Salmonella enterica serovar Typhi IgG	CCHF IgG	WNV IgG	Leptospira spp IgG	Francisella tularensis IgG	Bacillus anthracis IgG	Hantavirus IgM	Bartonella henselae/B quintana IgG	Borrelia spp IgG	Coxiella burnetii IgG
Adjara	42	3	6	0	2	1	4	3	4	4	0	0
Guria	15	1	4	0	3	1	0	0	1	0	0	0
Imereti	153	7	33	1	12	4	5	6	7	11	2	0
Kakheti	52	5	11	0	9	1	3	3	1	4	2	2
Kvemo Kartli	107	4	29	0	6	5	6	0	7	5	2	1
Mtskheta-Mtianeti	17	1	2	0	2	0	1	0	2	1	1	0
Racha-Lechkhumi Kvemo Svaneti	5	2	1	0	0	0	0	0	1	0	0	0
Samegrelo-Zemo Svaneti	95	4	15	0	15	3	1	4	7	4	1	0
Samtskhe-Javakheti	29	1	5	0	4	1	2	1	1	2	1	0
Shida Kartli	220	7	55	3	14	7	21	9	7	11	1	0
Tbilisi	205	15	65	5	12	5	10	4	18	12	1	3
Missing Region	60	1	7	1	8	0	8	2	3	3	2	0
Total	1,000	51	233	10	87	28	61	32	59	57	13	6

CCHF, Crimean-Congo hemorrhagic fever; IgG, immunoglobulin G; IgM, immunoglobulin M; WNV, West Nile virus.

Conclusion

In this study, immunological methods were used to detect seropositivity rates among a collection of military recruits for exposure to a select group of endemic diseases. It was particularly focused on describing the prevalence and distribution of newly emerging pathogens that have not been characterized previously among the military-age population. The results presented here indicate that there is a high degree of exposure among native-born Georgians that does not result in clinical illness. A more-thorough investigation will be necessary to determine the cause of this phenomenon; however, the evidence acquired during this study indicating widespread pathogen exposure suggests that military personnel operating in Georgia should take precautions to limit vector exposure and that the pathogens included in this study should be retained in the differential diagnosis of febrile and vector-borne illness in patients who are being treated in Georgia or those who have recently returned from a deployment to Georgia or a neighboring country.

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Author Contributions

NC carried out the experiment and helped supervise the project. NG, NR, and ND carried out the experiment. KD and LD contributed to the study design, enrollment, and sample collection. RK, PI, and MB contributed to the study design and helped supervise the project. RC contributed to the data entry and data analysis. TK and NA contributed to the study design and implementation and contributed to the manuscript writing. RR and MN provided devised the project and provided oversight. CB helped with data analysis and manuscript writing. MW helped supervise the project and provided major

support in manuscript writing. TA implemented the study as PI, contributed to the manuscript writing. All authors discussed the results and commented on the manuscript.

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