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14. ABSTRACT

The oligosaccharide galactose-α-1,3-galactose (α-Gal) is a blood group-like antigen of non-primate mammals and is the causal epitope in an IgE-mediated allergic disorder called the α-Gal syndrome. Ingestion of red meat and other products derived from mammals (e.g., dairy) can lead to allergic manifestations including hives, swelling, abdominal cramping, and anaphylaxis with a characteristic delay of 3-6 hours in subjects who are sensitized to α-Gal. An additional feature that distinguishes α-Gal syndrome from traditional food allergies is that sensitization to α-Gal is caused by tick bites, specifically bites of Amblyomma americanum (the lone star tick) in the United States. This study's purpose is to utilize the Department of Defense Serum Repository to investigate the prevalence and incident seroconversion of α-Gal specific IgE, the blood marker for α-Gal syndrome, in banked serum from active military personnel who were stationed at installations where the lone star tick is common (i.e., select bases in the Southeast and coastal Atlantic). Additionally, we will relate these findings with the clinical record of the service members over a ten-year time window to determine whether IgE to α-Gal is associated with reported allergic, gastrointestinal, cardiovascular symptoms of disease and other medical conditions. All regulatory approvals have been obtained and the study is awaiting shipping of serum samples from the DoD Serum Repository to University of Virginia.

15. SUBJECT TERMS

alpha-gal allergy, meat allergy

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The oligosaccharide galactose-α-1,3-galactose (α-Gal) is a blood group-like antigen of non-primate mammals and is the causal epitope in an IgE-mediated allergic disorder called the α-Gal syndrome. Ingestion of red meat and other products derived from mammals (e.g., dairy) can lead to allergic manifestations including hives, swelling, abdominal cramping, and anaphylaxis with a characteristic delay of 3-6 hours in subjects who are sensitized to α-Gal. An additional feature that distinguishes α-Gal syndrome from traditional food allergies is that sensitization to α-Gal is caused by tick bites, specifically bites of *Amblyomma americanum* (the lone star tick) in the United States. This study's purpose is to utilize the Department of Defense Serum Repository to investigate the prevalence and incident seroconversion of α-Gal specific IgE, the blood marker for α-Gal syndrome, in banked serum from active military personnel who were stationed at installations where the lone star tick is common (i.e., select bases in the Southeast and coastal Atlantic). Additionally, we will relate these findings with the clinical record of the service members over a ten-year time window to determine whether IgE to α-Gal is associated with reported allergic, gastrointestinal, cardiovascular symptoms of disease and other medical conditions

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Alpha-gal, meat allergy, lone star tick, α-Gal syndrome

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1: Prevalence and Incidence

1. Acquire regulatory approval and transfer samples to UVA allergy laboratory.
2. Conduct IgE assays and share data with investigators.
3. Analysis of data, presentation and manuscript preparation.

Specific Aim 2: Nested Case-Control

1. Conduct subsample IgE against 9 allergens and IgG levels ImmunoCAP assays and obtain blood type, and rickettsia antibodies and share data with investigators
    a. Reverse ABO blood type
    b. Conduct IgE Assays to Other Allergens
    c. Rickettsia IgG antibodies
2. Data analysis, manuscript presentation

Specific Aim 3: Analyze associations between α-Gal and medical co-morbidities
1. Acquire diagnostic codes associated with medical diagnoses, procedures and blood work from DoD
2. Analyze data for association between IgE to α-Gal and co-morbid diagnoses
3. Preparation of manuscript and public presentation

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Specific Aim 1: Prevalence and Incidence
1. Acquire regulatory approval and transfer samples to UVA allergy laboratory. Complete
2. Conduct IgE assays and share data with investigators.
Complete: all 6000 samples have been serotyped for alpha-gal IgE
3. Analysis of data and manuscript preparation.- Complete, manuscript of the baseline Prevalence when military members initialy joined is accepted for publication, and the Manuscript covering incidence of alph-gal seroconversion is under peer review for Publication.

Specific Aim 2: Nested Case-Control
1. Conduct IgE against 9 allergens and IgG ImmunoCAP assays and share data with investigators
    a. Reverse ABO blood type
    Under the initial plan we anticipated a possible 250 subjects with alpha-gal at the second sample and based the estimation of 250 cases matching at a ratio of 1:1 to 250 controls. Based on actual results we modified the case-control to 169 cases matched to 169 controls. For this match we forced an exact control case by military base then utilized a nearest neighbor Mahalanobis distance match based on other demographics such as age, sex, race, Branch of Uniformed Service and military rank. This provided 338 matched cases and controls. All 338 samples have been blood typed.
    b. Conduct IgE Assays to Other Allergens among the case:control- completed
    c. Evaluate for rickettsia antibodies in case: control group- completed

Data analysis and manuscript- analysis complete. A manuscript is for combined IgG levels is under review, Blood type abstract has been presented at American Academy of Allergy, Asthma and Immunology Annual Conference 2023, and the Rickettsia Antibody data will be presented at the 2024, American Academy of Allergy, Asthma and Immunology Annual Conference 2024. Manuscripts will continue to be submitted for peer review and publication.

Aim 3: Analyze associations between α-Gal and medical co-morbidities

1. Acquire diagnostic codes associated with medical diagnoses, procedures and blood work from DoD.
   Complete
2. Analyze data for association between IgE to α-Gal and co-morbid diagnoses.
   Completed based on initial diagnostic code categorization- did not find any associations
3. Preparation of manuscript and public presentation.
   Not completed as there were no significant associations or findings deemed worthy of presenting, a descriptive summary of healthcare finding may be submitted for presentation as a future conference

Methods

Using banked sera from the Department of Defense Serum Repository (DoDSR), this longitudinal serological study (a repeated cross-sectional analysis) was performed assessing IgE levels to alpha-gal in US active duty military personnel. The DoDSR collects excess serum from military members completing periodic and mandatory screening for HIV and is the largest bank of human serum in the world. Study participants were included if they were active-duty service members in the U.S. Army, Air Force, Navy, or Marine Corps whose first assignment following completion of basic training was at one of the following installations: Fort Liberty, North Carolina; Fort Leonard Wood, Missouri; Fort Campbell, Kentucky; Fort Knox, Kentucky; Marine Corps Base Quantico, Virginia; Marine Corps Base Camp Lejeune, North Carolina; Shaw Air Force Base, South Carolina; Little Rock Air Force Base, Arkansas; Naval Station Norfolk, Virginia; and Naval Station Newport, Rhode Island (Figure 1).

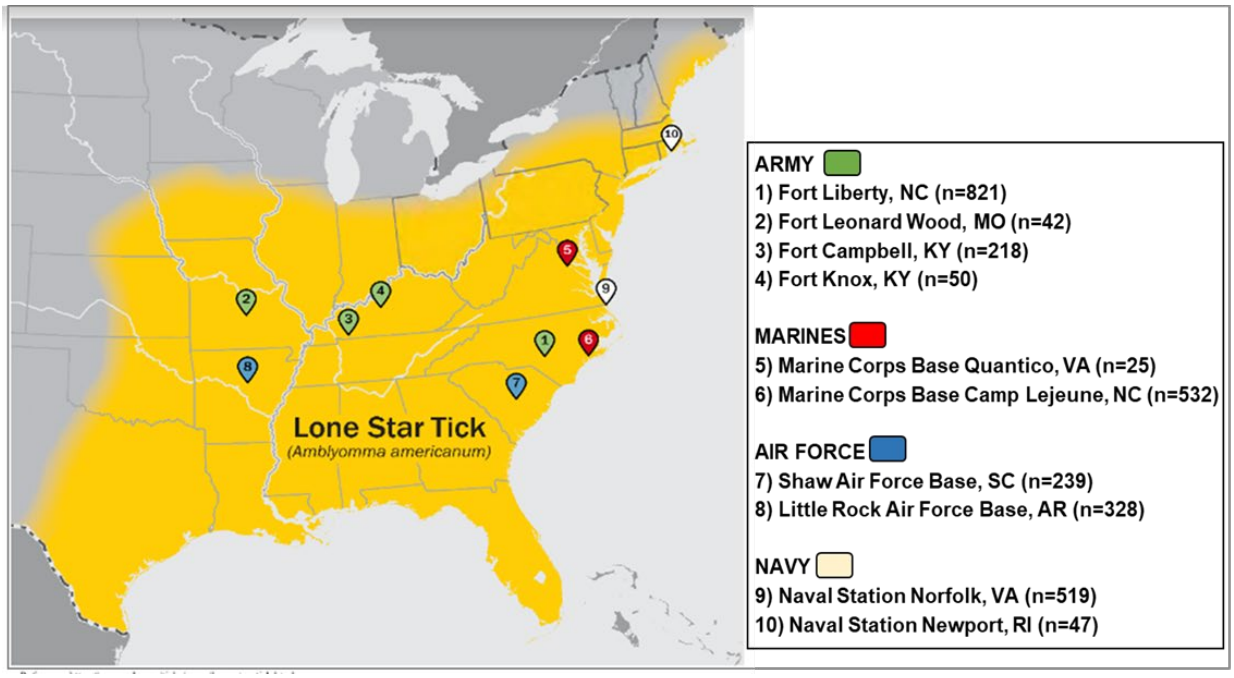


Figure 1 - Military installations investigated, in relation to the range of Amblyomma americanum, the lone star tick. Note, figure adapted from the approximate distribution of the Lone Star Tick from the Centers for Disease Control and Prevention, link: https://www.cdc.gov/ticks/maps/lone_star_tick.html

Samples were provided in a ratio of 1,200 Soldiers, 600 Airmen, 600 Sailors, and 600 Marines. Inclusion additionally required the availability of two serum samples while stationed at the same military installation. Samples were separated by at least 18 months and up to four years apart. The provided serum was collected in the DoDSR between the years of 2002 to 2008. The study excluded Reserve and National Guard service members on active duty.

Demographic data on de-identified subjects was reported from the DoDSR. Data included age in years, sex, self-reported race and ethnicity, rank, branch of service, and installation of assignment at the time of the serum draw. Rank was grouped as junior enlisted (cross service pay grade E1-E4), senior enlisted (E5-E9), junior officer (O1-O5), warrant officers, and others. The military branch of service was indicated as Air Force, Army, Marine Corps, or Navy, and the installation location was reported as the patient's location at the time the sera was obtained. Military occupations were grouped into categories, based on likely risk of exposure to ticks: administrative, artillery/ordinances, base support, construction and engineering, education and training, flight operations, infantry/law enforcement, and medical. The administrative group was chosen as the reference group. As many of the sera were collected upon entry into the military, military occupational code was based on the military occupation code assigned to the member at the time of their second serum draw.

Table 1 - Categories of Military Occupations

Occupational Category	**Occupation Title**
Administrative	General Administration, Automated Data Progressing (ADP) Computers, Analysis, Auditing and Accounting, Aviation Maintenance Records and Reports, Chaplain, Chaplain's Assistant, Combined Personnel and Administration, Comptroller and Fiscal Disbursing, Illustrating, Image Interpretation, Information and General Education, Intelligence, Intercept Operator, Interior Communications, Language Interrogation/Interpretation, Legal, Lithography, Manpower and Personnel, Meteorologist & Weather, Musician, General Operational Intelligence, Operations Staff, Operators/Analyst, Photography, Personnel, Procurement and Production, Recruiting and Counseling, Supply Administration
Artillery/Ordinances	Ammunition Repair, Armor and Amphibious, Artillery and Gunnery, Aviation Ordnance, Chemical, Explosive Ordnance Disposal/Underwater Demolition Team EOD/UDT, Ground and Naval Arms, Main Propulsion, Missile Artillery Operating Crew, Missile Fuel and Petroleum, Nuclear, Biological, and Chemical Warfare Specialist, Ordnance, Rocket Artillery, Small Arms Repair
Base Support	Food Service, Laundry and Personal Service, Logistics, Postal, Supply, Utilities, Warehousing and Equipment Handling, Sales Store, Teletype and Cryptographic, Equipment

Construction and Engineering	Automotive, Auxiliaries, Boatswain, Combat Engineering, Combat Operations Control, Construction and Utilities, Construction Equipment, Construction Equipment Operation, General Construction, Electric Power, Electrical/Electronic, Electricians, Electronic Instruments, Fabric, Leather and Rubber, Lineman, Machinist, Metal Body Repair, Motor Vehicle Operators, Other Mechanical and Electrical Equipment, Seamanship, Surveying, Tracked Vehicles Transportation, Welding, Woodworking
Education and Training	Cadets and Other Officer Candidates, Students, Military Training Instructor, Non-occupational Other, Not Occupationally Qualified, Undesignated Occupations
Flight Operations	Air Crew, Air Traffic Control, Air Traffic Control Radar, Aircraft Accessories, Aircraft Engines, Aircraft Launch Equipment, Aircraft Structures, General Aircraft, Aviation Maintenance and Allied, Communications and Radar, Communications Radio, Firefighting and Damage Control, Flight Operations, Forward Area Equipment Support, Helicopter Pilot, Navigation, Communications and Countermeasure, Non-Code Radio, Radar, Radio Code
Infantry/Law Enforcement	Corrections, Infantry, Investigation, Law Enforcement, Police, Security Guards, Small Arms Repair, Special Forces
Medical	Behavioral Sciences/Mental Health Services, Bioenvironmental Engineering, Biomedical Laboratory Services, Comprehensive Dentistry, Dental Care, Diet Therapy, Emergency Medicine, Endodontics, Environmental Health Services, Environmental Health/Preventive Medicine Services, Expeditionary Medical Services, Family Practice, Functional Analysis, Gastroenterology, General Dentistry, Nursing, General Surgery, Health Services Administration, Internal Medicine, Medical Administration, Medical Care and Treatment, Medical Logistics, Medical/Surgical Nurse, Operating Room Services, Oral Maxillofacial Surgery, Pharmacy, Physical and Occupational Therapy, Physiology, Diagnostic Radiology, Social Scientists, Veterinarian & Veterinary Medicine

Baseline prevalence was based upon 3 digit zip code as recorded as home of record when entering the military. US regions were classified as Northeast, Midwest, West, or South according to US Census bureau criteria. Urban/rural status, expressed as percent of the population living in a rural area, was derived from year 2000 5-digit ZIP Code Tabulation Area (ZCTA) data from the US Census Bureau and aggregated within each 3-digit zip. Among the 2966 subjects where urban/rural data were available, each recruit was assigned a home of record rural score, and the cohort was divided into roughly equal quintiles. Quintiles were used as a continuous variable in modeling. To generate a granular map of alpha-gal sensitization, we assessed alpha-gal IgE prevalence for each 3-digit zip code based on the US Census Bureau year 2000 ZCTA boundaries. To account for a wide variation in the number of recruits between 3-digit zip codes (with many having 0 or 1 recruit), we first explored manually grouping zip codes into

clusters of roughly 20 recruits each in the state of Virginia. We then expanded this analysis to the entire United States in a systematic fashion by assembling clusters of contiguous 3-digit ZCTAs using the Build Balanced Zones tool from the Spatial Statistics toolbox of ArcGIS Pro. Roughly 20 recruits were targeted per cluster while prioritizing compactness and minimizing alpha-gal IgE prevalence variance within each cluster.12 Using this tool, the minimum number of recruits within a cluster was 12, whereas the maximum number was 49 (median:18; interquartile range [IQR]: 16-21), and the assigned prevalence value of a cluster was expressed as a percentage of the total number of recruits in that cluster. Maps of tick distribution were generated using Center for Disease Control (CDC) data displaying county-level established A. americanum (lone star tick), Dermacentor variabilis (American dog tick), and Ixodes scapularis (deer or blacklegged tick) populations. A map of Ehrlichia chaffeensis infection cases (Ehrlichiosis) was generated using CDC data from the years 2016-2019. For each tick map, binary county-level data indicating the presence or absence of each tick within the county was rasterized using ArcGIS. Each 3-digit ZCTA was then assigned to be positive if a county within its boundary had an established tick population. The binary scores for the 3-digit ZCTAs within the aforementioned ArcGIS-generated clusters were then averaged, and Pearson's r was calculated to assess the correlation between the prevalence of alpha-Gal IgE and the average cluster value assigned to each tick. For Ehrlichiosis, the same process was performed; however, the value for each 3-digit ZCTA was assigned to be the maximum number of Ehrlichiosis cases from a county within the ZCTA, and these values were averaged among the 3-digit ZCTAs within each cluster.

Total IgE and alpha-gal specific IgE were measured by ImmunoCAP. Alpha-gal specific IgE was considered positive at a cut-off of greater than or equal to 0.1 kU/L, consistent with the Council of State and Territorial Epidemiologists Position Statement, 21-ID-07, Standardized Case Definition for Alpha-Gal Syndrome guidelines for confirmatory laboratory evidence of AGS (2022). Seroconversion was defined as an alpha-gal IgE level <0.1 kU/L at baseline that was ≥0.1 kU/L at the follow-up blood draw.

138 individuals had sero-converted from baseline and another 31 increased in alpha-gal IgE levels from baseline to follow-up. To further evaluate possible explanations for seroconversion a nested matched case-cohort was created among these 169 subjects and others who were neither positive at baseline nor seroconverted upon second sample analysis. Matching was performed using a combination of forced exact matching based on the assigned military installation (geography) and Mahalanobis distance nearest neighbor for the variables age, sex, race and ethnicity, branch of military service, and military rank. R 4.3.1 (R Core Team (2022). R A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.) and package matchit package was used for matching (Daniel E. Ho, Kosuke Imai, Gary King, Elizabeth A. Stuart (2011). MatchIt: Nonparametric Preprocessing for Parametric Causal Inference. Journal of Statistical Software, Vol. 42, No. 8, pp. 1-28. URL http://www.jstatsoft.org/v42/i08/). Additional statistical analysis was conducted using SAS v9.4 (Cary, NC), GraphPad Prism 9 (GraphPad Software, San Diego, Calif), R software, version 4.1.2 (R Foundation for Statistical Computing,Vienna, Austria), Datawrapper.de (Datawrapper GmbH, Berlin,Germany), and ArcGIS Pro, version 3.1.0 (ESRI, Redlands, Calif). . Frequencies with percentages and medians with interquartile ranges (IQR) were calculated for all variables overall and by alpha-gal conversion status. Other continuous variables were compared using the Student's t test or the Wilcoxon rank-sum test, as appropriate. Categorical data were compared using the chi2 test, and correlations were

assessed by calculating Pearson's r. Unadjusted and adjusted logistic regression was used for modeling seroprevalence at baseline. Wilcoxon rank-sum and Kruskal-Wallis tests were used for comparisons of IgE levels by demographic category. Because every subject had different times between serum samples, incidence densities were calculated for each demographic group using the number who seroconverted together with person time between the two samples collected. Poisson regression was used to estimate rate ratios (RR) and 95% confidence intervals (95% CI) of seroconversion. Unadjusted models were run for all studied variables. Due to strong correlation between occupation, installation, and branch of service, three separate adjusted models were run, including only one of each of these exposures while adjusting for demographic variables. The Uniformed Services University Institutional Review Board reviewed and approved this project.

Results:

Alpha-Gal Cohort Baseline Characteristics

Of the 3000 subjects, 2456 (81.9%) were male, median age was 19 (IQR 18-22) and most were junior enlistees (n=2840 [94.7%]) (Table 2). In regards to racial representation, 1957 (65.2%) subjects were White, 424 (14.1%) Black, 336 (11.2%) Hispanic, 135 (4.5%) Asian or Pacific Islander, 56 (1.9%) Native American and 92 (3.1%) other/unknown. Despite nine of the ten bases being in the southeast and coastal Atlantic, home of record spanned all 50 states, with a median of 36 recruits per state (range 3-261) (Table 3, Figure 2). Stratified by region, 415 (13.8%) had a home of record in the northeast, 627 (20.9%) from the Midwest, 1347 from the south (44.9%) and 561 (18.7%) from the West; 1200 (40%) were presenting to an Army base, 600 (20%) to a Naval base, 600 (20%) to an Air Force base and 600 (20%) to a Marine base. The number of subjects at individual bases ranged from 26 (Marine Corps Quantico) to 871 (Ft. Bragg), with a median of 241 (IQR [50-501]).

Table 2. Characteristics of the cohort at baseline

Characteristic	Overall Study Population	Alpha-Gal Present (≥0.1 IU/mL)	No Alpha-Gal (<0.1 IU/mL)	P-value
N	3000	179	2821	NA
Age, years (IQR)	19 (18-22)	19 (18-21)	19 (18-22)	0.11[a]
Sex				0.004[b]
Male	2456 (81.9%)	161 (89.9%)	2295 (81.4%)	
Female	544 (18.1%)	18 (10.1%)	526 (18.6%)	
Race				<0.001 [b]

White	1957 (65.2%)	147 (82.1%)	1810 (64.2%)	
Black	424 (14.1%)	11 (6.1%)	413 (14.6%)	
Hispanic	336 (11.2%)	8 (4.5%)	328 (11.6%)	
Native American (Z)	56 (1.9%)	6 (3.4%)	53 (1.9%)	
Asian/Pacific Islander (A+$I_{[WJM*1]}$)	90 +45 (4.5%)	5+1 (3.4%)	85+44 (4.6%)	
Other/unknown	92 (3.1%)	4 (2.2%)	88 (3.1%)	
Rank				0.64 [b]
Junior Enlisted	2840 (94.7%)	170 (94.9%)	2670 (94.6%)	
Senior Enlisted	21 (0.7%)	0 (0%)	21 (0.7%)	
Junior Officer	115 (3.8%)	8 (4.5%)	107 (3.8%)	
Other	24 (0.8%)	1 (0.6%)	23 (0.8%)	
Base				0.82 [b]
Ft. Bragg, NC	871 (29.0%)	50 (27.9%)	821 (29.1%)	
Ft. Leonard Wood, MO	44 (1.5%)	2 (1.1%)	42 (1.5%)	
Ft. Campbell, KY	230 (7.7%)	12 (6.7%)	218 (7.7%)	
Ft. Knox, KY	55 (1.8%)	5 (2.8%)	50 (1.8%)	
Marine Corps Base Quantico, VA	26 (0.9%)	1 (0.6%)	25 (0.9%)	
Camp Lejeune, NC	574 (19.1%)	42 (23.5%)	532 (18.9%)	
Shaw Air Force Base, AR	252 (8.4%)	13 (7.3%)	239 (8.5%)	
Little Rock Air Force Base, AR	348 (11.6%)	20 (11.2%)	328 (11.6%)	
Naval Station Norfolk, VA	552 (18.4%)	33 (18.4%)	519 (18.4%)	
Naval Station Newport,	48 (1.6%)	1 (0.6%)	47 (1.7%)	

RI				
Service				0.58 [b]
Army	1200 (40%)	69 (38.5%)	1131 (40.1%)	
Marines	600 (20%)	43 (24.0%)	557 (19.7%)	
Air Force	600 (20%)	33 (18.4%)	567 (20.1%)	
Navy	600 (20%)	34 (19.0%)	566 (20.1%)	
Home Residence[c]				<0.001
Northeast	415 (13.8%)	11 (6.1%)	404 (14.3%)	
Midwest	627 (20.9%)	42 (23.5%)	585 (20.7%)	
South	1347 (44.9%)	108 (60.3%)	1239 (43.9%)	
West	561 (18.7%)	16 (8.9%)	545 (19.3%)	
Other[d,]	50 (1.7%)	2 (1.1%)	48 (1.7%)	
Total IgE, IU/mL (IQR)	52 (19-159)	165 (57-411)	49 (18-144)	<0.001

[a] Student's T Test; [b] Chi-squared; [c] Regions defined according to US Census Bureau; [d] Includes Guam, US Virgin Islands, Puerto Rico, Armed Forces Europe and no home of record

Table 3 Demographics by Home State of Residence

Supplemental Table 3: Demographics by State / US Territory				Sex		Race				
State / Territory	Recruits, n	Sensitized, n (%)	Mean Age	Men, n (%)	Women, n (%)	White, n (%)	Black, n (%)	Hispanic, n (%)	Other, n (%)	Mean Rural Score
AK	18	3 (17%)	21.8	14 (78%)	4 (22%)	15 (83%)	1 (6%)	0 (0%)	2 (11%)	52
AL	42	4 (10%)	20.3	35 (83%)	7 (17%)	23 (55%)	18 (43%)	0 (0%)	1 (2%)	47
AR	36	14 (39%)	21.1	31 (86%)	5 (14%)	27 (75%)	7 (19%)	1 (3%)	1 (3%)	50
AZ	53	2 (4%)	19.7	43 (81%)	10 (19%)	37 (70%)	2 (4%)	7 (13%)	7 (13%)	15
CA	241	6 (2%)	20.4	182 (76%)	59 (24%)	112 (46%)	25 (10%)	61 (25%)	43 (18%)	9
CO	52	0 (0%)	21.0	45 (87%)	7 (13%)	38 (73%)	1 (2%)	8 (15%)	5 (10%)	16
CT	24	2 (8%)	20.5	20 (83%)	4 (17%)	14 (58%)	5 (21%)	4 (17%)	1 (4%)	15
DC	3	0 (0%)	19.7	1 (33%)	2 (67%)	1 (33%)	2 (67%)	0 (0%)	0 (0%)	0
DE	5	0 (0%)	20.2	5 (100%)	0 (0%)	5 (100%)	0 (0%)	0 (0%)	0 (0%)	33
FL	222	12 (5%)	20.4	173 (78%)	49 (22%)	129 (58%)	35 (16%)	39 (18%)	19 (9%)	11

GA	104	6 (6%)	20.4	85 (82%)	19 (18%)	47 (45%)	47 (45%)	4 (4%)	6 (6%)	33
GU	15	0 (0%)	20.3	12 (80%)	3 (20%)	1 (7%)	0 (0%)	0 (0%)	14 (93%)	N/A
HI	12	1 (8%)	22.3	9 (75%)	3 (25%)	5 (42%)	1 (8%)	1 (8%)	5 (42%)	15
IA	24	1 (4%)	20.8	23 (96%)	1 (4%)	20 (83%)	0 (0%)	1 (4%)	3 (13%)	43
ID	19	1 (5%)	20.2	14 (74%)	5 (26%)	18 (95%)	0 (0%)	1 (5%)	0 (0%)	30
IL	106	1 (1%)	20.2	86 (81%)	20 (19%)	74 (70%)	16 (15%)	6 (6%)	10 (9%)	16
IN	70	4 (6%)	21.1	61 (87%)	9 (13%)	58 (83%)	4 (6%)	2 (3%)	6 (9%)	28
KS	25	5 (20%)	20.2	24 (96%)	1 (4%)	23 (92%)	1 (4%)	1 (4%)	0 (0%)	28
KY	42	6 (14%)	21.3	36 (86%)	6 (14%)	36 (86%)	2 (5%)	2 (5%)	2 (5%)	41
LA	70	2 (3%)	20.7	56 (80%)	14 (20%)	45 (64%)	22 (31%)	2 (3%)	1 (1%)	31
MA	54	1 (2%)	20.4	47 (87%)	7 (13%)	40 (74%)	2 (4%)	5 (9%)	7 (13%)	10
MD	42	2 (5%)	21.9	35 (83%)	7 (17%)	21 (50%)	14 (33%)	3 (7%)	4 (10%)	18
ME	12	0 (0%)	19.4	10 (83%)	2 (17%)	11 (92%)	0 (0%)	0 (0%)	1 (8%)	55
MI	78	0 (0%)	20.2	68 (87%)	10 (13%)	58 (74%)	9 (12%)	4 (5%)	7 (9%)	30
MN	33	3 (9%)	20.8	28 (85%)	5 (15%)	28 (85%)	0 (0%)	2 (6%)	3 (9%)	31
MO	69	20 (29%)	21.4	59 (86%)	10 (14%)	58 (84%)	4 (6%)	2 (3%)	5 (7%)	45
MS	39	5 (13%)	21.4	30 (77%)	9 (23%)	21 (54%)	14 (36%)	1 (3%)	3 (8%)	51
MT	13	1 (8%)	19.8	12 (92%)	1 (8%)	12 (92%)	0 (0%)	0 (0%)	1 (8%)	46
NC	153	19 (12%)	20.6	121 (79%)	32 (21%)	97 (63%)	34 (22%)	12 (8%)	10 (7%)	42
ND	5	0 (0%)	20.2	5 (100%)	0 (0%)	4 (80%)	1 (20%)	0 (0%)	0 (0%)	47
NE	18	1 (6%)	20.1	16 (89%)	2 (11%)	16 (89%)	0 (0%)	1 (6%)	1 (6%)	40
NH	12	0 (0%)	20.9	11 (92%)	1 (8%)	10 (83%)	1 (8%)	1 (8%)	0 (0%)	46
NJ	55	2 (4%)	20.7	42 (76%)	13 (24%)	25 (45%)	7 (13%)	15 (27%)	8 (15%)	7
NM	16	0 (0%)	20.2	13 (81%)	3 (19%)	9 (56%)	0 (0%)	3 (19%)	4 (25%)	17
NV	16	0 (0%)	20.6	12 (75%)	4 (25%)	10 (63%)	2 (13%)	2 (13%)	2 (13%)	15
NY	142	1 (1%)	20.8	112 (79%)	30 (21%)	80 (56%)	24 (17%)	23 (16%)	15 (11%)	19
OH	143	6 (4%)	20.2	123 (86%)	20 (14%)	113 (79%)	16 (11%)	4 (3%)	10 (7%)	24
OK	20	7 (35%)	20.2	18 (90%)	2 (10%)	14 (70%)	1 (5%)	1 (5%)	4 (20%)	43

Table 3 continued				**Sex**		**Race**				
State / Territory	**Recruits, n**	**Sensitized, n (%)**	**Mean Age**	**Men, n (%)**	**Women, n (%)**	**White, n (%)**	**Black, n (%)**	**Hispanic, n (%)**	**Other, n (%)**	**Mean Rural Score**
OR	28	1 (4%)	21.5	25 (89%)	3 (11%)	22 (79%)	0 (0%)	2 (7%)	4 (14%)	22
PA	105	5 (5%)	20.1	90 (86%)	15 (14%)	89 (85%)	3 (3%)	6 (6%)	7 (7%)	33
PR/VI	21	2 (10%)	22.6	16 (76%)	5 (24%)	0 (0%)	4 (19%)	16 (76%)	1 (5%)	6
RI	9	0 (0%)	19.0	7 (78%)	2 (22%)	7 (78%)	1 (11%)	1 (11%)	0 (0%)	13
SC	79	5 (6%)	19.8	58 (73%)	21 (27%)	50 (63%)	24 (30%)	1 (1%)	4 (5%)	42
SD	9	0 (0%)	20.0	6 (67%)	3 (33%)	7 (78%)	0 (0%)	0 (0%)	2 (22%)	43
TN	67	7 (10%)	21.0	54 (81%)	13 (19%)	47 (70%)	14 (21%)	2 (3%)	4 (6%)	35
TX	261	9 (3%)	20.6	225 (86%)	36 (14%)	147 (56%)	24 (9%)	73 (28%)	17 (7%)	20
UT	21	0 (0%)	23.4	18 (86%)	3 (14%)	18 (86%)	0 (0%)	3 (14%)	0 (0%)	16
VA	134	10 (7%)	20.6	109 (81%)	25 (19%)	81 (60%)	26 (19%)	7 (5%)	20 (15%)	24

VT	3	0 (0%)	23.0	2 (67%)	1 (33%)	3 (100%)	0 (0%)	0 (0%)	0 (0%)	80
WA	59	1 (2%)	20.9	50 (85%)	9 (15%)	48 (81%)	3 (5%)	2 (3%)	6 (10%)	18
WI	52	1 (2%)	19.9	39 (75%)	13 (25%)	46 (88%)	2 (4%)	1 (2%)	3 (6%)	40
WV	23	0 (0%)	23.0	21 (91%)	2 (9%)	18 (78%)	4 (17%)	0 (0%)	1 (4%)	46
WY	13	0 (0%)	20.8	12 (92%)	1 (8%)	8 (62%)	0 (0%)	2 (15%)	3 (23%)	34
Other	13	0 (0%)	21.5	7 (54%)	6 (46%)	11 (85%)	1 (8%)	1 (8%)	0 (0%)	N/A

* DC – District of Columbia, GU – Guam, PR/VI – Puerto Rico and US Virgin Islands

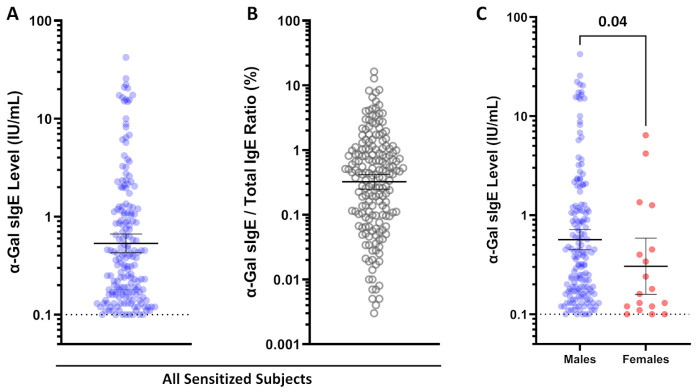


Figure 2. Geometric mean (GM) (A) alpha-gal IgE levels and (B) alpha-gal IgE/Total IgE ratios (expressed as a percentage) among all military personnel who tested positive for alpha-gal IgE and (C) GM alpha-gal IgE levels among sensitized males and females. Dotted black lines (A and C) indicates positivity threshold (≥0.1 IU/mL).

Baseline alpha-gal sensitization

Alpha-gal IgE ≥0.1 IU/mL was detected in 179 (6.0%) of the 3000 subjects in the initial blood draw. Age was similar among the sensitized and non-sensitized, but sensitization was twice as common in men (6.6%) as compared to women (3.3%), P=0.004. Differences in race were observed between sensitized and non-sensitized groups (P<0.001) with the following sensitization rates: White – 7.5%, Black 2.6%, Hispanic 2.4%, Native American 10.7%, Asian/Pacific Islander 4.4% and 4.3% for other race. Alpha-gal sensitization at intake was similar regardless of rank or branch of military service. Consistent with other reports, alpha-gal sensitization was more common among subjects whose home of record was in the South (8.0%), intermediate in the Midwest (6.7%) and less common among personnel coming from the northeast (2.7%) or west (2.9%). Total IgE was higher among sensitized vs non-sensitized subjects (GM 165 IU/mL vs 49 IU/mL, P<0.001). Among the positive subjects, the median alpha-gal IgE level was 0.38 kU/L (IQR 0.1-1.2). This was

significantly lower than in a reference cohort of patients we previously reported who had symptomatic AGS, suggesting many of the individuals in the current cohort may not have had symptomatic AGS (Figure 2).

Alpha-gal sensitization by state and smaller geographic clusters

By state, the highest prevalence rates were in Arkansas (39%), Oklahoma (35%) and Missouri (29%) (Supp Table 1 and Fig 1). States with intermediate prevalence included Alaska (17%), Kentucky (14%), Mississippi (13%), North Carolina (12%), Tennessee (10%), Alabama (10%), Minnesota (9%), Hawaii (8%) and Virginia (7%). There were no cases from Utah, West Virginia, Colorado or Michigan and less than 2% in Wisconsin, Massachusetts, Washington, Illinois and New York. (Figure 3)

The focused mapping of Virginia revealed that cases were widespread in the lower-elevation Piedmont and Coastal Plain regions, but there were few cases in the more urban Virginia Beach area, or in the mountainous western regions of the state. In the Virginia 3 digit zip code clustered map, sensitization prevalence values ranged from 0% to 24% within each cluster. (Figure 4B). Strikingly, only one of the 71 recruits (1.4%) from the more urban regions of the state, such as Northern Virginia and Virginia Beach (clusters 1, 2, and 3), was sensitized, while 9 of the 63 recruits (14.3%) from the other areas of the state were sensitized, $p<0.001$.

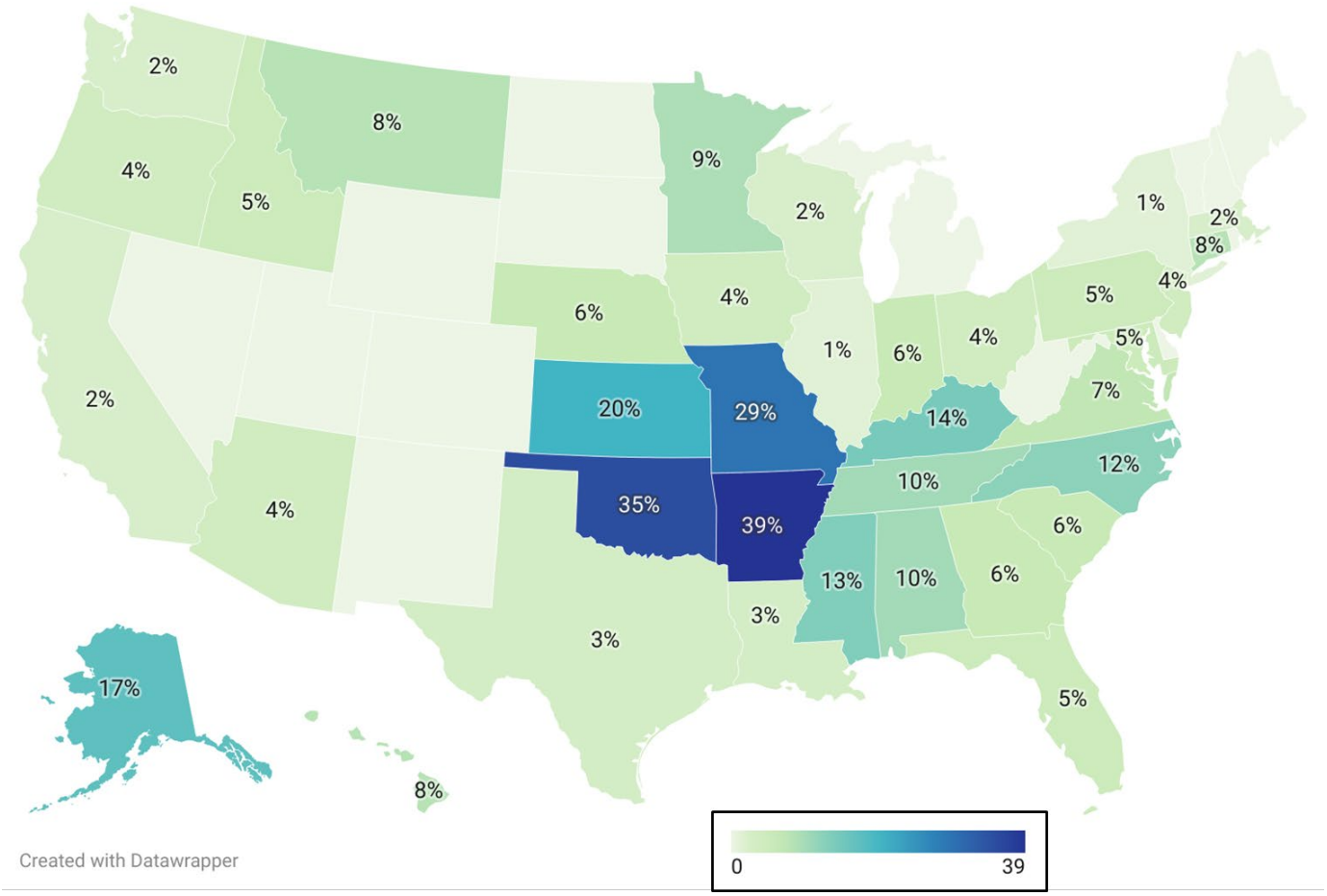
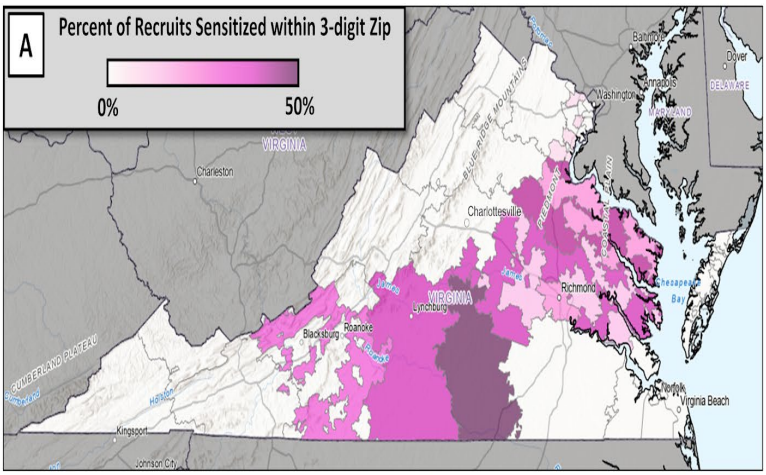


Figure 3. Alpha-gal IgE prevalence by state. States in light grey did not have any subjects with positive alpha-gal IgE.



Cluster No.	Location	No. Recruits	No. Sensitized	Percent Sensitized
1	*Virginia Beach and Eastern Shore*	25	0	0%
2	*Norfolk, Hampton and Southeastern Virginia*	20	0	0%
3	*Northern Virginia*	26	1	4%
4	*Richmond, Eastern Piedmont and Coastal Plain*	22	4	18%
5	*Central Virginia and Piedmont*	21	5	24%
6	*Shenandoah Valley and Western Virginia*	20	0	0%

Figure 4: A) Prevalence of alpha-gal IgE in the 28 3-digit zip codes in Virginia (of note, some 3-digit zip codes include non-contiguous areas). The number of recruits per 3-digit zip ranged from 0 to 19, with prevalences ranging from 0% to 50%. B) Manually grouped clusters of 3-digit zip codes in Virginia with approximately 20 recruits per cluster, and prevalence ranging from 0% to 24% per cluster.

Exploration of alpha-gal sensitization by race, sex and urban/rural status

To investigate factors associated with alpha-gal sensitization we carried out logistic regression modeling. Female sex, Black or Hispanic race and home residence outside of the south all had an inverse relationship with sensitization in models accounting for age, sex, race and home of record. To systematically explore effects of urban vs. rural living, rural scores within 3-digit ZCTAs were used to separate the cohort into rural/urban quintiles (Figure A5). Subjects residing in rural areas had significantly higher sensitization rates than their counterparts in more urban areas (Figure 5B; 12.5% rural vs. 3.7% urban, P<0.001).

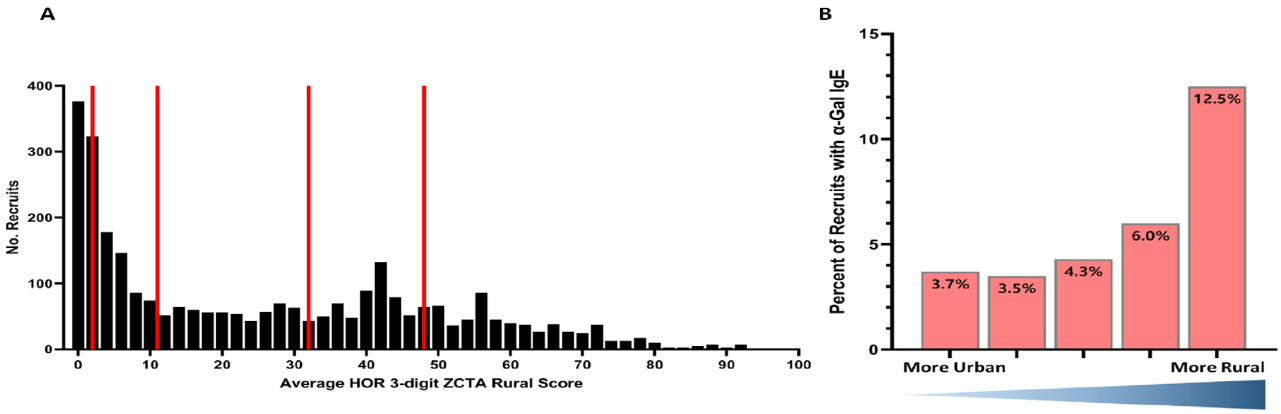


Figure 5. Urban/rural status of participants. A) Each 3-digit ZCTA Urban/Rural Score was ascertained from US Census Bureau Population data, and the cohort was stratified into quintiles. B) Alpha-gal sensitization rates in relation to quintiles of urban/rural residents in a ZCTA.

The inverse association of female sex, non-White race and home residence outside of the south on alpha-gal sensitization persisted in models accounting for urban/rural status (Table 4).

Table 4. Univariate and multiple variable models of alpha-gal sensitization

Characteristics	Unadjusted OR (n=3000)	Model #1 aOR (n=3000)	Model #2 aOR (n=2966)
Age[a]	0.96 [0.91-1.01]	0.95 [0.90-1.00]	0.96 [0.90-1.01]
Sex			
Male	2.05 [1.25-3.37]	1.69 [1.02-2.80]	1.76 [1.04-2.96]
Female	ref	ref	ref
Race			
White	ref	ref	ref
Black	0.33 [0.18-0.61]	0.29 [0.16-0.55]	0.30 [0.15-0.58]
Hispanic	0.30 [0.15-0.62]	0.32 [0.16-0.67]	0.40 [0.18-0.86]
Native American (Z)	0.70 [0.22-2.27]	0.74 [0.23-2.41]	0.81 [0.25-2.69]
Asian/Pacific Islander (A+I)	0.57 [0.25-1.32]	0.75 [0.32-1.76]	0.91 [0.39-2.13]
Other/unknown	0.56 [0.20-1.55]	0.58 [0.21-1.61]	0.68 [0.24-1.92]
Home Residence[b]			
Northeast	0.93 [0.43-2.02]	0.89 [0.41-1.95]	0.80 [0.36-1.76]
Midwest	2.42 [1.35-4.36]	2.12 [1.17-3.83]	1.77 [0.97-3.23]
West	ref	ref	ref
South	2.98 [1.75-5.09]	3.21 [1.87-5.51]	2.61 [1.51-4.53]
Other[c]	1.45 [0.32-6.49]	2.12 [0.46-9.73]	6.08 [0.68-54.5]
Urban/Rural Status[a,d]	1.48 [1.31-1.66]	-	1.33 [1.18-1.51]

Odds ratio (OR) with [95% CI] in logistic regression modeling
Model #1: adjusted for age, sex, race and region of home state
Model #2: adjusted for age, sex, race, home region and urban/rural status of 3-digit HOR ZCTA
[a] Incorporated into model as a continuous variable; [b] Regions defined according to US Census Bureau; [c] Includes Guam, US Virgin Islands, Puerto Rico, Armed Forces Europe and no home of record; [d] Urban/Rural status derived from US Census Bureau 3-digit ZCTA population data (available for n=2966) and incorporated in the model as continuous quintiles

Alpha-gal prevalence in relation to tick populations

Tick bite status was not known for this study, however to explore relationships between ticks and alpha-gal sensitization we compared our granular alpha-gal sensitization map to maps created using CDC data on the distribution of three major North American ticks – *A. americanum, D. variabilis and I. scapularis*. The intra-cluster correlation between our prevalence map and the A. americanum map (Figure 6B) was moderately strong with a correlation coefficient of r=0.41, p<0.001. By contrast, the correlation with the *D. variabilis* map was lower at r=0.17, p=0.04 and the correlation with *I. scapularis* was similarly low at r=0.23, p=0.005.. As an alternative approach to investigate the relevance of A. americanum, we also compared the alpha-gal map with the map of Ehrlichiosis cases. Of note, Ehrlichiosis is a reportable infection in which the relevant bacteria *Ehrlichia chaffeensis* is dominantly transmitted by A. americanum. The correlation between the alpha-gal sensitization map and the Ehrlichiosis map (Figure 6C) was relatively strong with a correlation coefficient of r=0.55, p<0.001.

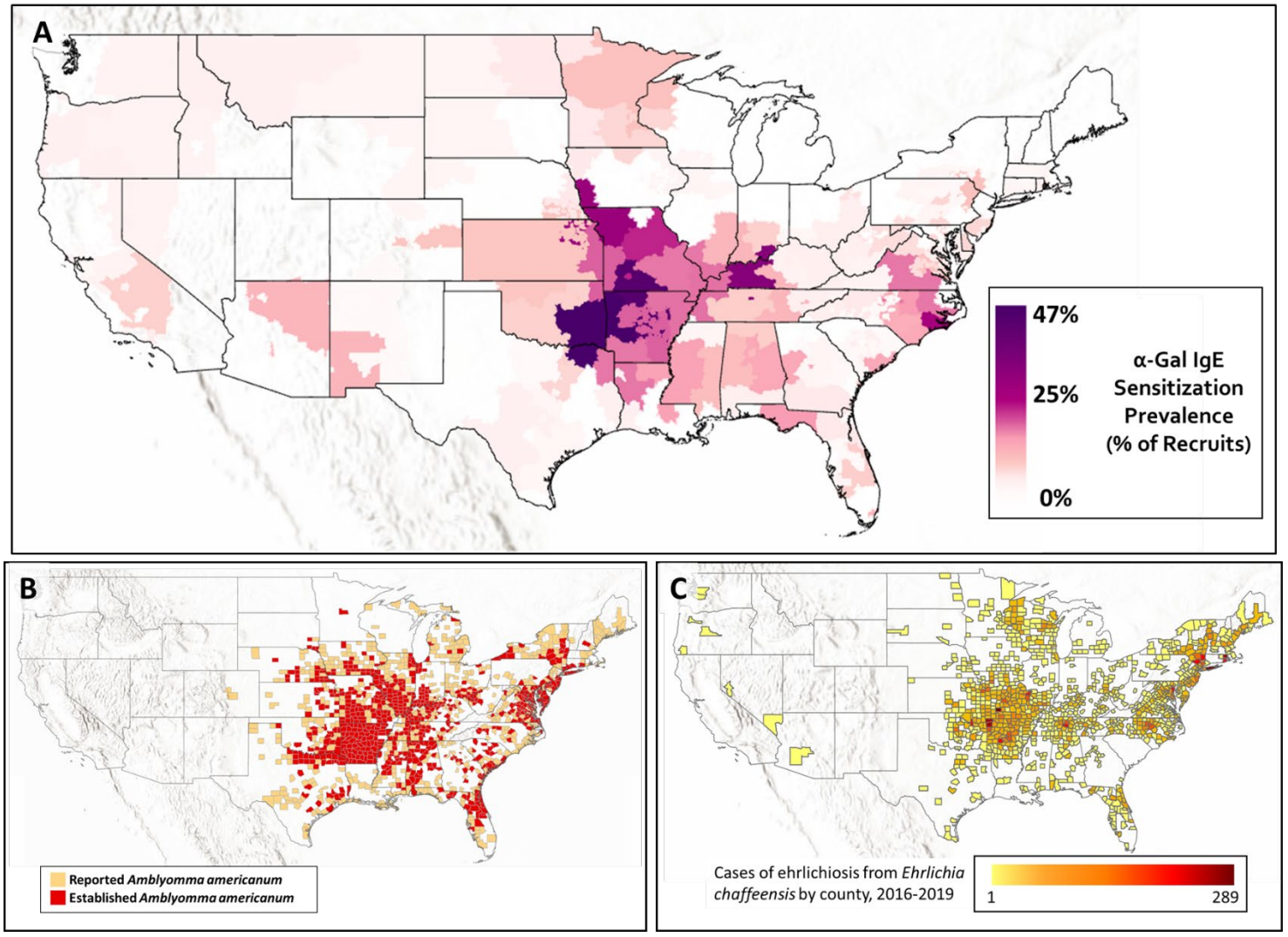


Figure 6. A) Patterns of alpha-gal IgE prevalence using 3-digit zip codes to assemble automated clusters of ~20 recruits each, B) County-level distribution of *A. americanum* ticks, C) County-level counts of *E. chaffeensis* infections.

Seroconversion to Alpha-gal IgE

Of the 3,000 subjects in the original study, all had their first and second serum samples successfully assayed and the median time between the first and second samples was 3.42 years (interquartile range [IQR], 3.19-3.68 years). The baseline prevalence of alpha-gal IgE was 179 (6.0%) among service members at the time of first assignment to one of the selected military installations. The results of the analysis on samples from military members who tested positive at baseline have been previously described and were excluded from further reporting or analysis[19]. Among the 2,821 who were negative for alpha-gal IgE at the baseline serum analysis, the median age was 19 (IQR 18-22), the majority were male (81.4%) and self-identified as White (64.2%), and most were junior enlisted rank (94.7%; Table 5). As was defined by the study design, a majority of subjects were Army service members (40%) with about 20% equally in the Air Force, Marines, and Navy. The administrative (23%), infantry/law enforcement (22.3%), and flight operations (22.7%) groups were the most common, followed by construction and engineering (12.6%) artillery/ordinance (7.4%), medical (5.5%), base support (5.1%), and education/training (1.3%).

Table 5- Population Demographics for the 2,821 Military Members Negative for Alpha-gal IgE at Baseline

Characteristic	Number (percent within each category)
Total	2821
Median age in years (IQR)	19 (18-22)
Sex	
Male	2295 (81.4%)
Female	526 (18.7%)
Race and Ethnicity	
White	1810 (64.2%)
Black	413 (14.6%)
Hispanic	328 (11.6%)
Native American	44 (1.6%)
Asian/Pacific Islander	85 (3.0%)
Other/Unknown	141 (5.0%)
Rank	
Junior Enlisted	2670 (94.7%)
Senior Enlisted	21 (0.7%)
Junior Officer	107 (3.8%)
Other	23 (0.8%)
Occupational Category	
Administrative	650 (23.0%)
Artillery/Ordinances	209 (7.4%)
Base Support	145 (5.1%)
Construction and Engineering	356 (12.6%)
Education and Training	36 (1.3%)
Flight Operations	641 (22.7%)
Infantry/Law Enforcement	629 (22.3%)
Medical	155 (5.5%)
Branch	
Air Force	567 (20.1%)
Army	1131 (40.1%)
Marines	557 (19.7%)
Navy	566 (20.1%)
Installation	
Fort Liberty, NC	821 (29.1%)
Fort Leonard Wood, MO	42 (1.5%)
Fort Campbell, KY	218 (7.7%)
Fort Knox, KY	50 (1.8%)
Marine Corps Base Quantico, VA	25 (0.9%)
Camp Lejeune, NC	532 (18.9%)
Shaw Air Force Base, AR	239 (8.5%)
Little Rock Air Force Base, AR	328 (11.6%)

Naval Station Norfolk, VA	519 (18.4%)
Naval Station Newport, RI	47 (1.7%)

Alpha-gal IgE seroconversion occurred in 138 service members (4.9%; Table 6). The median alpha-gal IgE level for those who seroconverted was 0.39 (IQR 0.19-1.01). There were differences in levels of alpha-gal IgE by sex and race and ethnicity but not rank, base, branch of service or occupation (Table 7). Seroconversion frequency and incident density were over three times higher in men than women (P<0.001). Differences in race and ethnicity were evident, with seroconversion less common in Blacks (1.0%) and Hispanics (1.5%) than Whites (6.6%) or Asian/Pacific Islanders (7.1%) (P<0.001). There was no difference in seroconversion by rank (P=0.149). The Army had the highest proportion of seroconversion (8.3%) then Air Force (3.7%), Marines (2.9%), and Navy (1.2%), respectively (P<0.001). There were significant differences in seroconversion between military installations (P<0.001), with the highest absolute number of cases at Fort Liberty, North Carolina (n=76; 9.3%) although the largest percentage of members who seroconverted were at Fort Leonard Wood, Missouri (14.3%) followed by Marine Corps Base Quantico, Virginia (12.0%). Seroconversion was most common in the infantry/law enforcement occupational category (12.7%) and lowest in the administrative category (1.2%; P<0.001).

Table 6 - Population Demographics by Seroconversion Incidence Status

	Seroconversion N and percent within the same category that seroconverted N=138	Total Person-Years	Seroconversion Incidence Density (per 1000 person years)	P value
Sex				<0.001
Male	128 (5.6%)	7908.1	16.2	
Female	10 (1.9%)	1813.5	5.5	
Race and Ethnicity				<0.001
White	119 (6.6%)	6234.8	19.1	
Black	4 (1.0%)	1427.3	2.8	
Hispanic	5 (1.5%)	1126.0	4.4	
Native American	1 (2.3%)	155.7	6.4	
Asian/Pacific Islander	6 (7.1%)	292.0	20.5	
Other/Unknown	3 (2.1%)	485.9	6.2	
Rank				0.149
Junior Enlisted	132 (4.9%)	9200.9	14.3	
Senior Enlisted	3 (14.3%)	69.9	42.9	
Junior Officer	2 (1.9%)	372.6	5.4	
Other	1 (4.4%)	78.4	12.8	
Occupational Category				<0.001
Administrative	8 (1.2%)	2246.0	3.6	
Artillery/Ordinances	6 (2.9%)	714.5	8.4	
Base Support	4 (2.8%)	487.1	8.2	
Construction and Engineering	13 (3.7%)	1218.2	10.7	
Education and Training	2 (5.6%)	122.8	16.3	
Flight Operations	21 (3.3%)	2235.0	9.4	
Infantry/Law Enforcement	80 (12.7%)	2153.2	37.2	
Medical	4 (2.6%)	544.9	7.3	
Branch				<0.001
Air Force	21 (3.7%)	1972.3	10.6	
Army	94 (8.3%)	3876.4	24.2	
Marines	16 (2.9%)	1901.5	8.4	
Navy	7 (1.2%)	1971.6	3.6	
Installation				<0.001
Fort Liberty, NC	76 (9.3%)	2827.1	26.9	
Fort Leonard Wood, MO	6 (14.3%)	139.0	43.2	
Fort Campbell, KY	8 (3.7%)	734.2	10.9	
Fort Knox, KY	4 (8.0%)	176.1	22.7	

Marine Corps Base Quantico, VA	3 (12.0%)	84.9	35.3	
Camp Lejeune, NC	13 (2.4%)	1816.6	7.2	
Shaw Air Force Base, AR	2 (0.8%)	836.4	2.4	
Little Rock Air Force Base, AR	19 (5.8%)	1135.9	16.7	
Naval Station Norfolk, VA	6 (1.2%)	1806.4	3.3	
Naval Station Newport, RI	1 (2.1%)	165.1	6.1	

Table 7- Median and Interquartile Range of Alpha-gal IgE Levels by Demographic and Occupational Categories Among Seroconverted Subjects

	Seroconversion Number	Alpha Gal IgE (kU/L [IQR])	P-value
All Combined	138	0.39 (0.19-1.01)	
Sex			0.02[a]
Male	128	0.40 (0.20-1.06)	
Female	10	0.17 (0.13-0.41)	
Race and Ethnicity			0.02[b]
White	119	0.48 (0.21-1.07)	
Black	4	0.12 (0.11-0.17)	
Hispanic	5	0.41 (0.15-1.91)	
Native American	1	0.91 (0.91-0.91)	
Asian/Pacific Islander	6	0.13 (0.12-0.14)	
Other/Unknown	3	0.31 (0.19-0.38)	
Rank			0.33[b]
Junior Enlisted	132	0.39 (0.19-1.06)	
Senior Enlisted	3	0.14 (0.14-0.54)	
Junior Officer	2	0.19 (0.13-0.25)	
Other	1	0.91 (0.91-0.91)	
Base			0.52[b]
Camp Lejeune, NC	13	0.36 (0.21-0.48)	
Fort Liberty, NC	76	0.53 (0.21-1.43)	
Fort Campbell, KY	8	0.34 (0.14-0.55)	
Fort Knox, KY	4	0.25 (0.18-0.82)	
Fort Leonard Wood, MO	6	0.46 (0.14-2.02)	
Little Rock Air Force Base, AR	19	0.27 (0.13-1.79)	
Naval Station Newport, RI	1	0.53 (0.53-0.53)	
Naval Station Norfolk, VA	6	0.24 (0.13-0.55)	
Marine Corps Base Quantico, VA	3	0.98 (0.14-19.50)	
Shaw Air Force Base, SC	2	0.19 (0.19-0.19)	
Occupational Category			0.13[b]
Administrative	8	0.27 (0.12-0.54)	
Artillery/Ordinances	3	1.05 (0.36-1.83)	
Base Support	4	0.31 (0.16-0.51)	
Construction and Engineering	16	0.24 (0.16-0.54)	
Education and Training	2	0.36 (0.12-0.60)	
Flight Operations	21	0.21 (0.14-0.78)	
Infantry/Law Enforcement	80	0.53 (0.22-1.51)	
Medical	4	0.26 (0.19-1.03)	
Service			0.41[b]
Army	94	0.49 (0.19-1.38)	
Marines	16	0.39 (0.19-0.60)	

Air Force	21	0.24 (0.19-0.78)	
Navy	7	0.30 (0.13-0.55)	

[a] Wilcoxon rank sum test; [b] Kruskal-Wallis test

Seroconversion rate ratios were calculated and unadjusted RR aligned closely with the results of seroconversion frequency and incident density (Table 8). In unadjusted analysis females had a lower rate of seroconversion (RR, 0.34 95% CI, 0.18-0.65). Compared to White personnel, Black (RR, 0.15; 95% CI, 0.05-0.40) and Hispanic (RR 0.23; 95% CI, 0.10-0.57) personnel had lower rates of seroconversion. Personnel working in construction and engineering (RR, 3.00; 95% CI ,1.24-7.23) and infantry/law enforcement (RR 10.43 95% CI, 5.04-21.58) had higher rates than personnel in administration. Rates were highest at Fort Leonard Wood, Missouri and Marine Corps Base Quantico, Virginia as compared to Shaw Air Force Base, South Carolina. Unadjusted and adjusted rate ratios including demographics and occupations are presented in Table 9. In adjusted analysis, differences in race and ethnicity, but not age, sex or rank, persisted in the fully adjusted models. The higher rate of seroconversion among personnel in construction and engineering and infantry/law enforcement also persisted, as did differences between the military installations.

Table 8 - Unadjusted and Adjusted Rate Ratios including Demographics and Occupation for Alpha-gal IgE Seroconversion

	Unadjusted Rate Ratio (95% CI)	Adjusted Rate Ratio (95% CI)
Age*	1.05 (1.01 -1.09)	1.04 (0.98-1.09)
Sex		
Male	ref	ref
Female	0.34 (0.18-0.65)	0.82 (0.41-1.63)
Race and Ethnicity		
White	ref	ref
Black	0.15 (0.05-0.40)	0.26 (0.09-0.72)
Hispanic	0.23 (0.10-0.57)	0.34 (0.14-0.83)
Native American	0.34 (0.05-2.41)	0.48 (0.07-3.51)
Asian/Pacific Islander	1.08 (0.47-2.44)	1.54 (0.67-3.53)
Other/Unknown	0.32 (0.10-1.02)	0.54 (0.17-1.73)
Rank		
Junior Officer	ref	ref
Junior Enlisted	2.67 (0.66-10.80)	2.24 (0.51 - 9.90)
Senior Enlisted	8.00 (1.34-47.86)	2.90 (0.44 - 19.32)
Other	2.38 (0.22-26.21)	2.29 (0.20 - 26.43)
Occupational Category		
Administrative	ref	ref
Artillery/Ordinances	2.36 (0.82 – 6.79)	1.85 (0.64 - 5.41)
Base Support	2.31 (0.69 - 7.66)	2.34 (0.70 - 7.82)
Construction and Engineering	3.00 (1.24 – 7.23)	2.46 (1.01 - 6.03)
Education and Training	4.57 (0.97 - 21.54)	4.06 (0.86 - 19.27)
Flight Operations	2.64 (1.17 - 5.96)	2.27 (0.998 - 5.16)
Infantry/Law Enforcement	10.43 (5.04 - 21.58)	7.05 (3.31 - 15.02)
Medical	2.06 (0.62 - 6.84)	2.20 (0.64 - 7.55)

*Rate ratio represents one year increase in age

Nested case-control study

Rickettsia IgG

Among the 138 incident alpha-gal cases, median age was 23.4, 128 were male (93%) and 119 were white (86%). The largest number of recruits who seroconverted to IgE alpha-gal (n=76 [55%]) were from Fort Liberty, NC, which exceeded the proportion from Ft. Liberty in the parent cohort (n=871 [29%]), P<0.001. Age, sex, race and military base were similar among the 163 in the matched control group. The prevalence of IgG to SFGR was 26.8% in those who seroconverted and 12.9% in the controls (P=0.002).

Blood Type

(n=169) matched for age, sex and geography, incident alpha-gal subjects trended toward a lower frequency of B or AB blood types (12% vs 16%), but this was not significant (P=0.28).

Total IgE (tIgE)

Baseline tIgE was higher in these alpha-gal sensitized compared to non-sensitized subjects (GM 165 vs 49 IU/mL). Among the 2821 subjects not alpha-gal sensitized at baseline, 138 (4.9%) seroconverted during their service. Baseline tIgE levels were higher among those who seroconverted compared to those who did not (GM 86 vs 47 IU/mL). Among seroconverters, tIgE levels increased (median change 24 IU/mL, p<0.001), but did not increase among non-seroconverters. The increases in tIgE among were not accounted for by increases in IgE to alpha-gal (median change 0.38 IU/mL, p<0.001).

Other IgE allergen sensitization

Nine additional allergens were evaluated in the case-control group of alpha-gal seroconversion vs those who did not. IgE comparisons are shown in figure 7. There were significantly higher proportion of subjects positive for alpha-gal who also were positive for Peanut, Honey Bee, and Yellow Jacket IgE. This suggests a likely association between skin insect exposure from outdoor activities and allergy development among those with alpha-gal IgE.

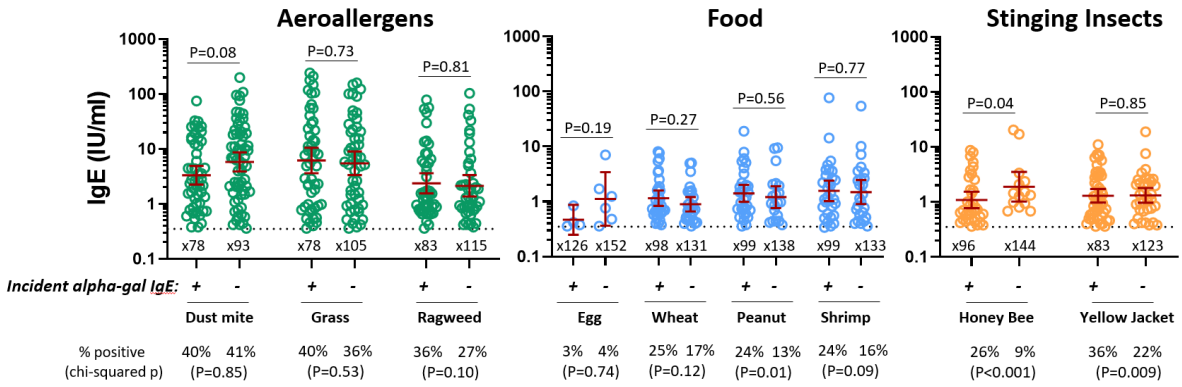


Figure 7 Aallergen specific IgE levels among those who did seroconvert to positive Alpha-Gal IgE (+)to those who did not (-)

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Research Mentorship and Training of Medical Student, Alex Noth.
Research Mentorship and Training of Allergy Fellow, Jamin Patel.
Training in laboratory technique, Sam Ailsworth.
Research Mentorship and Training of Preventive Medicine Resident, Susan Ching

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

An abstract with the prevalence and incidence has been submitted to and accepted as a poster presentation at the Military Health System Research Symposium in Orlando, FL planned in September 2022.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

This study is complete, we of course will continue to work to submit our manuscripts generated from this grant to peer review journals seeking publication.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

- Baseline prevalence is high among recruits from areas that overlap with the Lone Star tick and Rickettsia, supporting the association between positivity and tick bites.
- Seroconversion data demonstrated an adjusted male risk, higher risk among whites and Asian race, and higher occupational risks associated with outdoor training (infantry vs. admin or medical), there was a low risk among Navy personnel evaluated.
- Blood types B or AB have an insignificantly lower risk for the development of alpha-gal IgE.
- High level baseline IgE is a risk factor for the subsequent development of alpha-gal IgE, suggesting a predisposition for sensitization is likely a risk factor among some military members.
- Regarding Rickettsia antibodies, prevalence of IgG to spotted fever group Rickettsia was twice as high among military members who were sensitized to alpha-gal during the study period.
- The co-occurrence of insect allergy supports outdoor and tick exposure as source of alpha-ga

The key finding of our study may not had a large impact yet but as our results make through peer review and publication, the impact will be seen through novel epidemiological observations such as differences in race and sex. The high rate of seroconversion among service members, especially in occupations that have high exposure to outdoor training and work, suggests that military service and assignment to bases within the range of the Lone Star tick is a risk for the development of disqualifying conditions such as alpha-gal syndrome. This impact is potentially effects on individual and unit readiness and implications for service-connected medical condition for military members separated because of food allergies, specifically to meat. Also, the high level of seroconversion suggests that current preventive medicine interventions such as permethrin, DEET and proper pant tucking is not adequate to protect against the effects of tick bites and alpha-gal syndrome.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*

- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

One significant deviation from the study design was differences in the proportion of samples from each base. We requested equal number of samples from each base and we received from the DoD Serum Repository the following samples.

Base	Actually Sampled
Ft Bragg, NC	871
Ft. Leonard Wood	44
Ft. Cambell, KY	230
Ft. Knox, KY	55
Quantico, VA	26
Camp Lejeune, NC	574
Shaw AFB, SC	252
Little Rock, AR	348
Norfolk, VA	552
Naval Station, RI	48

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

There was significant delay in acquiring the serum from the DoD serum repository as a result of the COVID-19 pandemic. This obstacle was able to be overcome with a one year no-cost extension and we have completed the study.

The serology sensitization subtask against lone star tick was substituted for *Rickettsia amblyommii* sensitization (intended as a surrogate for tick exposure) at project negotiation based on the scientific reviewer suggestion. The process for measuring direct sensitization again the lone star tick is itself is experimental. We are acquiring lone star tick saliva from WRAIR in an attempt for this method but it may not produce expected results. Although these results would be interesting if successful, this part of the study is a non-essential portion for completion of the overall project aims.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

No changes had a significant impact on expenditures

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Jaimin Patel "Investigation into alpha-Gal sensitization patterns in military personnel" was presented at the 2022 UVA Swineford Allergy Conference (Charlottesville, VA)

Nylund CM, Susi A, Workman LJ, Platts-Mills T, Wilson J. Prevalence and Incidence of IgE Sensitization to the Mammalian Meat Allergen galactose-alpha-1,3- galactose in Active Duty Service Members. Military Health System Research Symposium, Kissimmee Florida. Sept 2022. *Poster*.

Wilson JM, Ailsworth S, Workman LJ, Patel J, Keshavarz B, Nelson M, Platts-Mills TAE, Susi A, Nylund CM. Alpha-Gal IgE prevalence and regional variability: investigation of 3000 Military Personnel. Gordon Food Allergy Conference Oxnard, CA. Nov 2022 Poster

Wilson JM, Ailsworth S, Workman LJ, Patel J, Keshavarz B, Nelson M, Platts-Mills TAE, Susi A, Nylund CM. Alpha-Gal IgE prevalence and regional variability: investigation of 3000 Military Personnel. 2023 American Academy of Allergy Asthma & Immunology Annual Meeting, San Antonio, TX. Feb 2023. Poster

Makin T, Workman LJ, Ailsworth S, Nylund CM, Borish L, Wilson JM, Lawrence M. Investigation into IgE Deficiency as a Predictive Marker of Hypogammaglobulinemia. American Academy of Allergy Asthma & Immunology Annual Meeting, San Antonio, TX. Feb 2023. Poster

Ailsworth S, Noth A, Patel J, Workman LJ, Keshavarz B, Nelson M, Platts-Mills TAE, Susi A Nylund CM Association of Sex and ABO-blood Group with Alpha-Gal Sensitization in a Cohort of Military Personnel. American Academy of Allergy Asthma & Immunology Annual Meeting, San Antonio, TX. Feb 2023. Poster

Ching SJ, Susi A, Workman LJ, Platts-Mills TAE, Wilson JM, Nylund CM. Occupational Risk Associated with IgE Seroconversion to the Mammalian Meat Allergen Galactose-alpha-1,3- galactose in US Active Duty Service Members. Military Health System Research Symposium, Kissimmee Florida. August 2023. *Platform*

Ailsworth SM, Workman LJ, Susi A, Platts-Mills TAE, Nylund CM, Wilson JM. Is high-level total IgE a risk factor for alpha-gal sensitization or a consequence of tick bites? American Academy of Allergy Asthma & Immunology Annual Meeting, 2024 Submitted.

Bortz PS, Susi A, Platts-Mills TAE, Nylund CM, Wilson JM. Prevalence of IgG to Rickettsia in a Cohort of Military Personnel in Relation to Incidence Alpha-Gal Sensitization. American Academy of Allergy Asthma & Immunology Annual Meeting, 2024 Submitted.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Ailsworth SM, Susi A, Workman LJ, Ji YS, Patel J, Nelson MR, Platts-Mills TAE, Nylund CM, Wilson JM. Alpha-gal IgE Prevalence Patterns in the United States: An Investigation of 3,000 Military Recruits. J Allergy Clin Immunol Pract. 2023 Nov 2:S2213-2198(23)01201-1. doi: 10.1016/j.jaip.2023.10.046. Epub ahead of print. PMID: 37918651.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding*

support is provided from other than this award.)

Name:	Cade Nylund
Organization:	Uniformed Services University of the Health Sciences
Project Role:	Principle Investigator
ORCID:	0000-0003-4543-6804
Contribution to Project:	Principle Investigator

Name:	Jeffrey Wilson
Organization:	University of Virginia
Project Role:	Co-Investigator/ UVA Site Director
ORCID:	0000-0002-5975-1760
Contribution to Project:	Co- Investigator, administrative preparation, preparing lab, ordered and set up lab: IgE machine and reagents.

Name:	Apryl Susi
Organization:	Henry Jackson Foundation
Project Role:	Co-Investigator
ORCID:	0000-0003-2580-2563
Contribution to Project:	Co-Investigator, coordination of IRB, contracts, DSSA, support agreements, healthcare record processing

Name:	Lisa Workman
Organization:	University of Virginia
Project Role:	Lab technician
ORCID:	
Contribution to Project:	Alpha-gal serum assays

Name:	Thomas Platts-Mills
Organization:	University of Virginia
Project Role:	Co-investigator
ORCID:	0000-0002-1263-329X
Contribution to Project:	Co-Investigator, expert consultation

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing of overlap to report during this study period.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organizational Name: The University of Virginia
Location of Organization: Charlottesville, VA
Partner's Contribution to the Project: Collaboration

8. **SPECIAL REPORTING REQUIREMENTS**
   COLLABORATIVE AWARDS:

9. **QUAD CHARTS:**

10. **APPENDICES:**

Prevalence and seroconversion of IgE to the mammalian oligosaccharide galactose-α-1,3-galactose (α-Gal) and relationship to comorbid disease in military personnel

TB180090

W81XWH-18-TBDRP-IIRA

PI: Cade Nylund, MD **Org:** Uniformed Services University of the Health Sciences **Award Amount:** 809,000

Study/Product Aim(s)

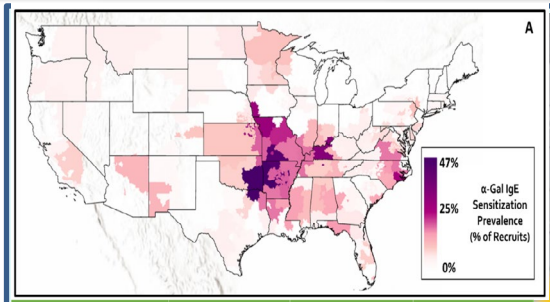
• : Determine prevalence and incidence of IgE to α-Gal in banked serum specimens from military personnel stationed at select sites where lone star ticks are endemic

• Compare α-Gal sensitization to prevalence of IgE & IgG seropositivity Rickettsia, B-group antigen, and atopy.

• Evaluate qualitative and quantitative associations between the presence of α-Gal specific IgE and clinical diagnoses as recorded in the Military Health System database.

Approach

Paired serum from 300 subjects at each of ten pre-determined active-duty military installations will be randomly selected by the Department of Defense Serum Repository for analysis to determine basic baseline prevalence and then subsequent incident seroconversion to α-Gal IgE. The results will also serve as the basis of a case-control analysis to assess for the relationship between α-Gal IgE status and other diagnoses or symptoms as documented in the medical record by interrogation of ICD-9 and ICD-10 codes.



	Nested Case-Control Analysis	α-Gal Incident Recruits	Matched controls	p
	B or AB blood group	20/169 (12%)	27/169 (16%)	0.28
Atopy	Atopic	100/159 (63%)	86/158 (54%)	0.13
Atopy	Positive to 1+ Inhalant	94/159 (59%)	77/158 (49%)	0.06
Atopy	Positive to 1+ Food	57/159 (36%)	30/158 (19%)	<0.001
Atopy	Positive to 1+ Venom	52/159 (33%)	29/158 (18%)	0.003

tary bases from which sera will be collected and investigated

Accomplishment:, 6000 samples serotyped for alpha-gal. subsample blood types, and other allergen IgE tested. Rickettisa antibodies measured. Analysis for baseline prevalence and incidence completed and submitted for publication.

Timeline and Cost

Activities CY	20	21	22	23
Acquire Regulatory Approval				
Conduct IgE Assays				
Analysis of Data				
Presentations, Manuscript Prep				
Estimated Budget ($K)	**$120**	**$500**	**$100**	**$89**

Updated: 23 Dec 2023

Goals/Milestones

CY19 Goal – Regulatory Approval

☑ IRB Approvals

☑ Finalize Support Agreements from DoD Serum Repository

☑ HRPO Approvals

☑ Data Sharing Agreement with DHA

CY20 Goal – Conduct Assays

☑ Transfer Serum to UVA

☑ Conduct IgE Assays for α-Gal

CY21 Goal – Conduct assays for nested case-control

☑ Assays for 9 allergens, and B blood group

CY22-2023 Goal

☑ IgG to Rickettsia

☑ Data analysis

☑ Manuscript drafting and submissions for publication (1 published, 1 under review)

Budget Expenditure to Date

Actual Expenditure: $ full amount, 809,000

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause) (See Instructions on back)

Form Approved
OMB No. 9000-0095
Expires Jan 31, 2008

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to the Department of Defense, Executive Services Directorate (9000-0095). Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE ABOVE ORGANIZATION. RETURN COMPLETED FORM TO THE CONTRACTING OFFICER.

1.a. NAME OF CONTRACTOR/SUBCONTRACTOR	c. CONTRACT NUMBER	2.a. NAME OF GOVERNMENT PRIME CONTRACTOR	c. CONTRACT NUMBER	3. TYPE OF REPORT (X one)
Uniformed Services University of the Health Sciences	CDMRPL-18-0-TB180090			☐ a. INTERIM ☒ b. FINAL

b. ADDRESS (Include ZIP Code)	d. AWARD DATE (YYYYMMDD)	b. ADDRESS (Include ZIP Code)	d. AWARD DATE (YYYYMMDD)	4. REPORTING PERIOD (YYYYMMDD)
4301 Jones Bridge Rd Bethesda, MD 20853	20190515			a. FROM b. TO

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME(S) OF INVENTOR(S) (Last, First, Middle Initial) a.	TITLE OF INVENTION(S) b.	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER c.	ELECTION TO FILE PATENT APPLICATIONS (X) d.				CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER (X) e.	
			(1) UNITED STATES		(2) FOREIGN			
			(a) YES	(b) NO	(a) YES	(b) NO	(a) YES	(b) NO
None	None	N/A						

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1) (a) NAME OF INVENTOR (Last, First, Middle Initial) N/A	(b) NAME OF EMPLOYER N/A	(1) TITLE OF INVENTION N/A	(2) FOREIGN COUNTRIES OF PATENT APPLICATION N/A
(b) NAME OF EMPLOYER	(c) ADDRESS OF EMPLOYER (Include ZIP Code)		
(c) ADDRESS OF EMPLOYER (Include ZIP Code)	(2) (a) NAME OF INVENTOR (Last, First, Middle Initial)		

SECTION II - SUBCONTRACTS *(Containing a "Patent Rights" clause)*

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME OF SUBCONTRACTOR(S) a.	ADDRESS (Include ZIP Code) b.	SUBCONTRACT NUMBER(S) c.	FAR "PATENT RIGHTS" d.		DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S) e.	SUBCONTRACT DATES (YYYYMMDD) f.	
			(1) CLAUSE NUMBER	(2) DATE (YYYYMM)		(1) AWARD	(2) ESTIMATED COMPLETION
University of Virginia	PO Box 801355, Charlottesville, VA 22908, USA	HU0001-22-2-0066	N/A	N/A	Serum IgA antibody levels for 6000 samples	20200201	20230822

SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR *(Not required if: (X as appropriate))* ☐ **SMALL BUSINESS or** ☒ **NONPROFIT ORGANIZATION**

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL (Last, First, Middle Initial)	b. TITLE	c. SIGNATURE	d. DATE SIGNED (YYYYMMDD)
Nylund, Cade M	Vice Chair for Research, Department of Pediatrics, School of Medicine, Uniformed Services University of the Health Sciences	NYLUND.CADE.MCCOY.1158030604 Digitally signed by NYLUND.CADE.MCCOY.1158030604 Date: 2023.12.28 11:40:04 -05'00'	20231228