AWARD NUMBER: CDMRPL-18-0-TB180090

TITLE: Prevalence and Seroconversion of IgE to the Mammalian Oligosaccharide Galactose-Alpha-1,3-Galactose and Relationship to Comorbid Disease in Military Personnel

PRINCIPAL INVESTIGATOR: Col Cade Nylund, MD

CONTRACTING ORGANIZATION: Uniformed Services University of the Health Sciences
Bld 61 Rm E227
4901 Jones Bridge Rd
Bethesda, MD 20853

REPORT DATE: AUGUST 2022

TYPE OF REPORT: Annual Technical Progress Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
AUGUST 2022	Annual	01 AUG 2021 - 31 Jul 2022

4. TITLE AND SUBTITLE

Prevalence and Seroconversion of IgE to the Mammalian Oligosaccharide Galactose-Alpha-1,3-Galactose and Relationship to Comorbid Disease in Military Personnel

5a. CONTRACT NUMBER
CDMRPL-18-0-TB180090

5b. GRANT NUMBER
CDMRPL-18-0-TB180090

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Cade Nylund, MD
Col, USAF, MC

E-Mail: cade.nylund@usuhs.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Uniformed Services University of the Health Sciences 4901 Jones Bridge Rd Bethesda, MD 20853

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	21	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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 and manuscript preparation.

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
    b. Conduct IgE Assays to Other Allergens
2. Data analysis and manuscript

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.
In progress

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 base then utilized a nearest neighbor Mahalanobis distance match based on other demographics such as age, sex, race, Uniformed Service and military rank. All 338 samples have been blood typed
    b. Conduct IgE Assays to Other Allergens
    Currently In progress
2. Data analysis and manuscript
    Not complete

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.
    Complete
3. Analyze data for association between IgE to α-Gal and co-morbid diagnoses.
    Not complete
4. Preparation of manuscript and public presentation.
    Not complete

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.

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.

The next steps will be completion of serology assays to determine IgE sensitization to other allergens. We initially intended to ascertain lone star tick exposure, as best as possible. This will be attempted the UVA team measuring lone star tick antibodies.

Additionally, we will seek to connect the alpha-gal serology results with the health care data for further analysis of associations.

Lastly, we will seek to collate our results for both presentations and publications.

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).

Nothing to Report, yet

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

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:*

Three major challenges have affected the timeline of our project by 18 months.

1. Worldwide pandemic of COVID-19
   The DoD serum to be utilized in for the study are supplied by the DoD Serum Repository which is managed by the Armed Forces Health Surveillance Branch (AFHSB). The AFHSB is the central epidemiologic resource for the U.S. Armed Forces, conducting medical surveillance for DoD members. With the onset of COVID-19 the AFHSB has had to shift resources away from routine studies to urgent COVID-19 surveillance. This delayed the transfer of serum samples. The samples have all been transferred.
2. Delay in obtaining the Data Sharing Agreement with the Defense Health Agency. Obtaining the DSAs simply took longer than anticipated. This has been resolved.
3. Delay in transfer of payment for serum samples to the AFHSB/DoD Serum Repository
   There was confusion over expectation of O&M funds vs. RDT&E funds and how to transfer the funds to AFHSB. This has been resolved.

One other discovered discrepancy in the research plan was that the DoD Serum Repository did not provide the serum samples in the planned ratio from each base. We requested 300 subjects selected from each base. We were provided much different ratios.

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

This is unfortunate but incidences can still be performed.

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.

Overall the study is currently delayed by 12 months. We anticipate we will be able to complete the work with the extension of a no-cost extension.

The serology sensitization subtask against lone star tick was substituted in place of *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.

A delay in meeting SOW milestones has led to less costs than anticipated at this point in the study. We anticipate utilizing these funds once the study proceeds.

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, Ebert ME, Susi A, Workman LJ, Platts-Mills T, Wilson JM. Prevalence and Incidence of IgE Sensitization to the Mammalian Meat Allergen galactose-alpha-1,3- galactose in Active Duty Service Members. *Accepted for presentation at the Military Health System Research Symposium, to be presented as poster September 2022.*

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).*

Nothing to Report

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".

Name:	Cade Nylund
Organization:	Uniformed Services University of the Health Sciences
Project Role:	Principle Investigator
ORCID:	0000-0003-4543-6804
Nearest Person-month worked:	4
Contribution to Project:	Principle Investigator

Name:	Jeffrey Wilson
Organization:	University of Virginia
Project Role:	Co-Investigator/ UVA Site Director
ORCID:	0000-0002-5975-1760
Nearest Person-month worked:	2
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
Nearest Person-month worked:	6
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:	
Nearest Person-month worked:	6
Contribution to Project:	Alpha-gal serum assays

Name:	Thomas Platts-Mills
Organization:	University of Virginia
Project Role:	Co-investigator
ORCID:	0000-0002-1263-329X
Nearest Person-month worked:	0.3
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.

New Awards:

Dr. Jeffrey Wilson

PI:
NIH R21, Award # 1R21AI166861-01 "Investigation of the prevalence, presentation and immunologic features of the α-Gal syndrome in a high-risk cohort not recruited on the basis of allergic disease" This is a different cohort and does not have overlap.

AI:
NIH R01, Award # 1R01AI172112 "IgE antibody responses to the oligosaccharide galactose-alpha-1, 3-galactose (alphagal) in murine and human atherosclerosis" No overlap.

Dr. Cade Nylund

PI:
Military Research Royalty Funds Award for the project entitled "The Impact of the Emerging COVID-19 Pandemic on Short- and Long-Term Military and Family Health, Mental Health, and Force Readiness." This project has no scientific overlap.

United States Air Force, 711th Human Performance Wing Studies and Analysis for the project entitled, "Neonatal, Perinatal, and Pediatric Outcomes for Children of Female Flyers." There is no scientific overlap for this project.

DoD High Priority Research. "The Long-Term Effects and Prognosis of SARS-CoV-2 Infection in Active Duty Military and Family Members" There is no overlap

AI:
DoD High Priority Research "The Impact of Disparities in Access to Behavioral and Specialty Care for Injured and Ill Service Members and Their Families" There is no scientific overlap.

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: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

A. Copy of Accepted Abstract to the Military Health System Research Symposium, to be presented September 2022 in Orlando, FL
B. Map of the percent of military members positive for alpha-gal IgE at initial serology sample based on home of record data.
C. Map of the Number of Subjects Sampled and Incidence of Alpha-gal Sensitization.

Appendix A Accepted Abstract to MHSRS

Cade M. Nylund MD, James M. Ebert MPH, Apryl Susi MS, Lisa J. Workman BA,, Thomas A E Platts-Mills MD, Jeffrey M. Wilson MD

1. Uniformed Services University of the Health Sciences, Department of Pediatrics, Bethesda, MD
2. University of Virginia, Department of Medicine, Division of Allergy and Clinical Immunology Charlottesville, VA

Submit to: Military Exposures and Subsequent Long-term Outcomes breakout session

Title: Prevalence and Incidence of IgE Sensitization to the Mammalian Meat Allergen galactose-alpha-1,3- galactose in Active Duty Service Members

Introduction: The presence of serum IgE specific for the oligosaccharide galactose-alpha-1,3-galactose (alpha-gal) is causally-linked with the alpha-gal syndrome (AGS). AGS describes a spectrum of immediate or delayed hypersensitivity reactions to meat and other food and medicinal products derived from non-primate mammals. AGS symptoms can include urticaria, angioedema, or anaphylaxis along with gastrointestinal symptoms. Of unique occupational health interest to military service members, tick bites, particularly those from *Amblyomma americanum* (lone star tick), are an important cause of AGS. The large number of military bases within the geographic range of the lone star tick in the US combined with the occupational risk of field training places military members at unique risk for the development of AGS. Little is known about the prevalence of this disorder in the overall US population or in the military. Using DoD banked serum, we sought to evaluate the prevalence and incidence of alpha-gal IgE sensitization.

Materials and Methods: Using banked sera from the Department of Defense Serum Repository (DoDSR) we have initiated a cross-sectional study of 3000 service members at two time points at least 18 months apart. The first sera sample was obtained from military members whose initial assignment following training, was at select military bases within the established geographical range of the lone star tick between 2002 and 2007. The sampling design included 1200 soldiers, 600 airmen, 600 sailors, and 600 marines. The bases included: Ft. Bragg, NC; Ft. Leonard Wood, MO; Ft. Campbell, KY; Ft. Knox, KY; Marine Corps Base Quantico, VA; Marine Corps Base Camp Lejeune, NC; Shaw Air Force Base, SC; Little Rock Air Force Base, AR; Naval Station Norfolk, VA; and Naval Station Newport, RI. Selection of sex, race, or military occupation were at random. Rank was categorized by E1-E4 (Junior enlisted), E5-E9 (Senior enlisted, O1-O5 (Junior Officer), warrant officers and others. Reserve or National Guard service members on active duty were excluded. Total IgE and alpha-Gal specific IgE measurements were conducted using commercially available ImmunoCAP assays. The threshold of sensitivity for the total IgE assay is 2 IU/mL and for the specific alpha-gal assay is 0.1 IU/mL (1 IU = 2.4 ng). A threshold of an alpha-gal specific IgE of 0.1 IU/mL was used to define a positive test. Comparisons between groups were performed using the chi-square test,

and when any cell was less than 5, the Fisher exact test. The Wilcoxon Rank Sum Test was used to compare continuous variables between groups.

Results: Of the 3,000 subjects in the study, all 3,000 subjects have had the first serum sample assayed. Of those, 2456 subjects were male (81.87%). The distribution of subject races were 1957 (65.23%) white, 424 (14.13%) black, 336 (11.20%) hispanic, 90 (3%) asian/pacific islander, 45 (1.5%) Native American/Alaskan Native and 148 (4.9%) other/unknown. Most were junior enlisted (2840; 94.67%) and young; median (IQR) age at first sampling was 19 (18-22) years-old. The rank breakdown was 2840 junior enlisted, 21 senior enlisted, 115 junior officers, 1 warrant officer, and 23 others. The baseline prevalence of alpha-gal IgE >/= 0.1 IU/mL was 177 (5.9%) among service members at the time of first assignment to one of the selected bases. The time between the first and second sample was approximately 3.42 years (median, 1249 days; interquartile range[IQR]) 1166-1342.5). Of the 177 service members who were positive at baseline, 93 (52.54%) of them remained positive at follow-up testing. Of the 2,823 who tested negative at baseline, all service members had follow-up testing. Of these 2,823 service members, the development of IgE antibodies against alpha-gal (*seroconversion*) occurred among 138 service members (4.89%).Service members who developed alpha-gal IgE were of similar age, yet significantly older, (median, 20 years; IQR, 19-23) as those who did not (median, 19 years; IQR,18-22; P = 0.002). Males (127; 5.53%) had a higher rate of seroconversion compared to females (11; 2.09%; P<0.001). Of 1966 Junior enlisted that did not have alpha-gal IgE at baseline, 58 (2.95%) seroconverted. Of 732 senior enlisted without alpha-gal at baseline, 75 (10.25%) seroconverted; of 121 junior officers tested without alpha-gal at baseline, 4 (3.31%) seroconverted, and of 3 warrant officers, there was 1 seroconversion, and one rank listed as “Other” did not seroconvert (P < .0001). Service members in the Army (94/1129) had the highest seroconversion (P<.0001), followed in descending order by the Air Force (21/567), Marine Corps (16/559), and Navy (7/568). The development of IgE antibodies occurred more at Army and Marine bases (P<.0001); the number of subjects that seroconverted at each base, listed from highest to lowest (in percent) are: Ft. Leonard Wood (6/42 [14.29%]); Marine Corps Base Quantico(3/25 [12%]); Ft. Bragg (75/821 [9.14%]); Ft. Knox (4/50 [8%]); Little Rock Air Force Base (19/328 [5.79%]); Ft. Campbell (9/216 [4.17%]); Marine Corps Base Camp Lejeune (13/534 [2.43%]); Naval Station Newport, (1/47 [2.13%]); Naval Station Norfolk (6/521[1.15%]); Shaw Air Force Base (2/239 [0.84%]).

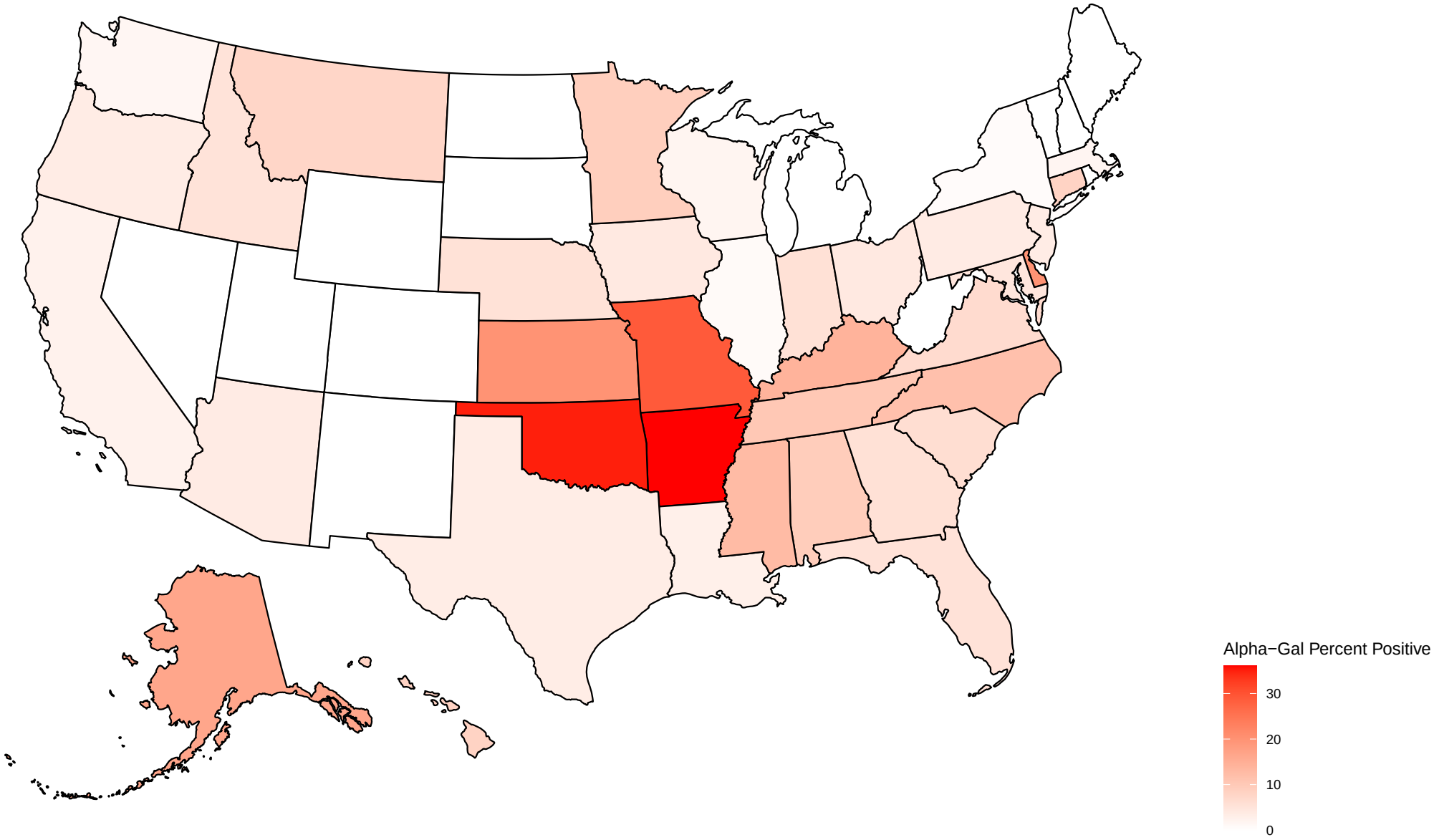
Conclusions: The baseline prevalence of sensitization to alpha-gal among these US service members was 5.9%, the majority ((52.5%) of whom maintained positivity at the follow-up time point. Also, there was a high incidence of seroconversion with alpha-gal specific IgE (4.9%). A higher seroconversion rate among soldiers and airmen/gaurdians vs. marines and sailors suggest a likely higher occupational risk (presumably from higher requirement for field exercises and thus exposure to the bite of the lone star tick). As AGS is a relatively newly recognized disorder with testing only recently becoming broadly available, it is likely that sensitized US service members who experienced symptoms would not have received a definite diagnosis or identification of an etiology at the time (sample mostly from early 2000s). Recognizing at-risk populations based on geography and occupational exposure to ticks can help with appropriate testing, diagnosis and treatment for service members presenting with symptoms consistent with AGS. Implications for military public health remain to be determined. Although questions remain about the full clinical spectrum associated with alpha-gal IgE, there is emerging evidence that

sensitization may expand risk beyond just allergy to mammalian meat. New data suggests associations with coronary artery disease and also with gastrointestinal morbidity. Further studies are needed to evaluate the full clinical impact of alpha-gal IgE sensitization on military readiness. Additional implications include a need for stricter enforcement of existing tick bite prevention and even questions around policy for service related illness. With anticipated completion of our study we will be able to inform a more complete understanding of alpha-gal sensitization in the military population.

Learning Objectives:

1. Recognize unique environmental occupational risks associated with military service and the development of alpha-gal syndrome and red meat allergy.
2. Describe the prevalence of alpha-gal sensitization among U.S young adults joining the military
3. Describe the incidence of developing IgE antibodies to alpha-gal in active duty service members.

Appendix B Map of Baseline Prevalence of Alpha-gal Sensitization Based on Home of Record



Appendix C

Incidence of Alpha−gal IgE Seroconversion at 10 Miltiary Bases