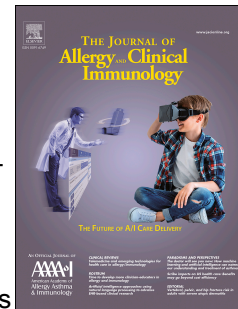


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PII: S0091-6749(20)30800-9

DOI: <https://doi.org/10.1016/j.jaci.2020.05.034>

Reference: YMAI 14601

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 7 February 2020

Revised Date: 21 April 2020

Accepted Date: 12 May 2020

Please cite this article as: Wilson JM, Keshavarz B, Retterer M, Workman LJ, Schuyler AJ, McGowan EC, Lane C, Kandeel A, Purser J, Ronmark E, LaRussa J, Commins SP, Merritt T, Platts-Mills TAE, A dynamic relationship between two regional causes of IgE-mediated anaphylaxis: α -Gal syndrome and imported fire ant, *Journal of Allergy and Clinical Immunology* (2020), doi: <https://doi.org/10.1016/j.jaci.2020.05.034>.

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A dynamic relationship between two regional causes of IgE-mediated anaphylaxis: α -Gal syndrome and imported fire ant

Jeffrey M. Wilson, MD, PhD^{†1}, Behnam Keshavarz, PhD^{†1}, Maya Retterer¹, Lisa J. Workman, BA¹, Alexander J. Schuyler, BS, BA¹, Emily C. McGowan, MD, PhD¹, Charles Lane, MD², Alaaddin Kandeel, MD³, Jane Purser, MD⁴, Eva Ronmark, PhD⁵, Joseph LaRussa, MD⁶, Scott P. Commins, MD, PhD⁷, Tina Merritt, MD⁸, Thomas A.E. Platts-Mills, MD, PhD^{*1}

Affiliations:

¹Division of Allergy & Clinical Immunology, University of Virginia, Charlottesville, VA.

²Allergy Partners of Lynchburg, Lynchburg, VA.

³Essentia Health, Duluth, MN.

⁴Allergy Clinic of Tulsa, Tulsa, OK.

⁵Department of Public Health and Clinical Medicine, OLIN Unit, Umea University, Umeå, Sweden.

⁶Pediatric and Adult Asthma and Allergy, Birmingham, AL.

⁷Division of Rheumatology, Allergy & Immunology, University of North Carolina, Chapel Hill, NC.

⁸The Allergy and Asthma Clinic of Northwest Arkansas, Bentonville, AR.

*Correspondence to: Thomas A.E. Platts-Mills, Division of Allergy & Clinical Immunology, University of Virginia, P.O. Box 801355, Charlottesville, VA, 22908-1355, USA
Tel: (434) 924-5917; Fax: (434) 924-5779; E-mail: tap2z@virginia.edu

† Indicates these authors contributed equally

Disclosures: TAE Platts-Mills and T. Merritt have a patent on an IgE assay to α -Gal and TPM has received assay support from Thermo-Fisher/Phadia; J. Wilson has received research support from Thermo-Fisher/Phadia; S. Commins has been on the speaker's bureau for Genentech. The remaining authors report no conflicts.

Funding: NIH R37 AI-20565 (TPM)

Word Count: 4813

ABSTRACT

Background: A syndrome of mammalian meat allergy relating to IgE specific for galactose- α -1,3-galactose (α -Gal) was first reported ten years ago in the southeastern United States and has been related to bites of the lone star tick (*Amblyomma americanum*).

Objective: Here we investigated the epidemiology of the “ α -Gal syndrome” in the USA and sought additional evidence for the connection to tick bites.

Methods: A survey of allergists was conducted using a snowball approach. A second tier of the survey included questions about anaphylaxis to imported fire ants (IFA). History of tick bites and tick-related febrile illness were assessed as part of a case-control study in Virginia. Antibody assays were conducted on sera from subjects reporting allergic reactions to mammalian meat or IFA.

Results: In North America the α -Gal syndrome is recognized across the Southeast, Midwest and Coastal Atlantic with many providers in this area each managing over 100 cases. The distribution of cases generally conformed to the reported range of *Amblyomma americanum*, although within this range there was an inverse relationship between α -Gal cases and cases of IFA anaphylaxis that were closely related to the territory of IFA. The connection between tick bites and α -Gal sensitization was further supported by questionnaire and serologic results of cases.

Conclusions: The α -Gal syndrome is commonly acquired in adulthood as a consequence of tick bites and has a regional distribution that largely conforms to the territory of the lone star tick. The epidemiology of the syndrome is expected to be dynamic and shifting north because of climate change and ecologic competition from IFA.

Key Messages

- The α -Gal syndrome is commonly diagnosed by allergists across the Southeast and also in parts of the Midwest and Coastal Atlantic in areas where the lone star tick is established
- A cluster of cases reported in northern Minnesota suggests the lone star tick is more prevalent here than previously reported or that other ticks and/or ectoparasites are relevant to sensitization in this area
- The reduced number of α -Gal cases in the Gulf Coast and Texas is likely explained by the presence of an ecologic competitor to ticks – the imported fire ant

Capsule Summary

The authors provide additional evidence for the connection between α -Gal and lone star ticks. A lower than expected number of α -Gal cases on the Gulf Coast is likely explained by competition from the imported fire ant.

Key words

Tick, galactose- α -1,3-galactose, mammalian meat allergy, α -Gal syndrome, IgE, anaphylaxis, imported fire ant

Abbreviations

α -Gal – galactose- α -1,3-galactose

CCD – cross-reactive carbohydrate determinant

ELISA – enzyme-linked immunosorbent assay

ICD – international classification of disease

IFA – imported fire ant

IgE – immunoglobulin E

IgG – immunoglobulin G

RMSF – Rocky mountain spotted fever

sIgE – specific IgE

SFG – spotted fever group Rickettsia

UVA – University of Virginia

INTRODUCTION

In 2007 a striking regional pattern of acute allergic reactions to the monoclonal antibody cetuximab was reported from centers in the southeastern USA¹. These reactions were found to be related to pre-existing IgE antibodies specific for the oligosaccharide galactose- α -1,3-galactose (α -Gal)^{2,3}. Using the assay for IgE to α -Gal to screen cases we identified a group of patients who had developed delayed urticarial or anaphylactic reactions to mammalian meat as adults⁴. The distribution of cases of these two forms of anaphylaxis, which are now both recognized as part of the “ α -Gal syndrome”, were noted to overlap with reported cases of Rocky Mountain Spotted Fever (RMSF). Although very few or none of the cases of mammalian meat allergy had a history of a febrile illness consistent with RMSF, subsequent investigation led to the identification of an association between bites from *Amblyomma americanum* (the lone star tick) and α -Gal cases^{5,6}. The understanding of the relationship to tick bites was further supported by monitoring subjects who developed IgE antibodies to α -Gal after experiencing tick bites and subsequently developed delayed reactions to mammalian meat^{5,7}. Cases of α -Gal syndrome, most of which have been ascribed to ticks, have now been described on six continents⁵⁻¹². Thus far, however, there has been little epidemiologic investigation of the syndrome in the United States and also few studies comparing α -Gal cases in different geographic areas. The connection with ticks has also raised several important questions, many of which relate to the mechanisms whereby tick bites can lead to the induction of IgE specific for α -Gal⁶.

Our goal here was to describe the epidemiology of cases of α -Gal syndrome in the USA and to relate the findings to the reported distribution of *A. americanum*. When it became clear that mammalian meat allergy cases were uncommon in the Gulf Coast states and Texas, despite the reported presence of the lone star tick in this area, we considered the possibility that the imported fire ant (*Solenopsis* spp) (IFA), an established competitor of *A. americanum*¹³⁻¹⁶, could be relevant to this discrepancy. We also compared immunologic features of the syndrome in subjects presenting with mammalian meat allergy from two distinct geographic areas, and exposure and tick bite history were compared between α -Gal sensitized and non-sensitized subjects. Finally, to address a hypothesis that bacteria present in ticks could be a relevant co-

111 factor in α -Gal sensitization, serum antibodies to tick symbionts were measured in subjects
112 reporting mammalian meat allergy.

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METHODS

Survey of α -Gal syndrome cases in the USA

The study was carried out by surveying allergists in practice starting in 2016 in an area of the Southeast with a high number of established α -Gal cases. Most surveys were conducted by telephone, however some provider questionnaires were completed in-person at local or national allergy meetings, or by email communications. The initial sampling included many allergists in practice known to the authors, however, participating allergists were asked about other providers in their region who might be seeing cases. These other providers were hence contacted and, thus, the survey was extended to most areas of the United States in a “snowball” fashion. Because traditional snowball sampling involves patient interviews, whereas here our approach was to survey providers, we have referred to this as a modified snowball technique^{17, 18}.

The questionnaire used by the surveyor was a single page sufficiently simple to be carried out in less than 10 minutes and focused on the number of α -Gal cases that had been confirmed by skin testing or serum IgE assay (see Fig E1). Categorization of cases seen in the last three years (0, 1-4, 5-40, 41-100, >100) was pre-determined prior to initiating the survey based on preliminary discussion with allergy colleagues in the Southeast. With allergists who reported seeing five or more cases we asked further questions about how they were confirmed and their response to dietary avoidance. Despite the reported presence of *A. americanum* in the Gulf Coast states and Texas it became clear during the initial survey that the cases of confirmed α -Gal were less than expected in these areas. Based on information about the geographical distribution and predatory activity of the IFA we conducted a second phase of the questionnaire (n=86 surveys) which inquired about the number of cases of fire ant anaphylaxis that had been seen and confirmed by allergy testing (see Fig E2). Fire ant cases were also categorized by the number seen in the last three years: 0, 1-4, 5-40, 41-100, >100.

Major Populations investigated

1. Central Virginia: As part of a previously reported IRB-approved case-control study at the University of Virginia, sera were available from 211 subjects with α -Gal syndrome¹⁹. These

cases were defined by self-report of allergic reactions to mammalian meat and a positive α -Gal specific IgE (sIgE) blood test. The cases were derived from a parent study which included subjects who reported allergic reactions to mammalian meat (n=261), subjects who reported allergic reactions without an obvious trigger (n=111) and control subjects without histories of hives or anaphylaxis (n=36). Within this parent study, 311 subjects had IgE to α -Gal ≥ 0.35 IU/mL and 97 had no detectable IgE to α -Gal (cut-off = 0.35 IU/mL). A detailed questionnaire, which included information about exposure history, including tick bites, was filled out by all participants.

2. “Three Corners”: Sera from de-identified subjects evaluated for suspected mammalian meat allergy was provided by clinicians from Bentonville, AR (n=50), Springfield, MO (n=23) and Tulsa, OK (n=31), which we collectively refer to as “Three Corners”. Of these, 75 tested positive with the IgE assay to α -Gal and were considered as α -Gal cases, and 29 tested negative.
3. Birmingham, AL: Sera from de-identified subjects evaluated for mammalian meat allergy (n=8) or fire ant anaphylaxis (n=24) was provided by a practice in Birmingham, AL. Cases of IFA anaphylaxis or α -Gal syndrome were defined by clinical history with positive skin and/or IgE blood testing.
4. Sweden: Subjects from Kiruna and Luleå in northern Sweden were enrolled in a prospective, population-based study as previously reported²⁰. The study included detailed information about asthma and exposure history and was approved by the regional ethics committee of Umeå University and the Human Investigation Committee at the University of Virginia. Of the 963 subjects who provided a blood sample at the age 19 year time point, 413 of these were selected for α -Gal sIgE testing. This included all 218 subjects who were sensitized to cat and an additional 195 randomly selected subjects.

IgE assays to allergens

Total and specific IgE antibodies were measured using commercially available ImmunoCAP assays (Thermo Fisher/Phadia, US, Portage, MI) performed with the ImmunoCAP 250 instrument, and the results were expressed as IU/mL (1 IU $\approx$ 2.4 ng of IgE)²¹. All assay results reported here were conducted in the research lab at UVA. The streptavidin CAP technique was

used to measure IgE to α -Gal using cetuximab on the solid-phase²². Subjects were considered to be sensitized to α -Gal if they had IgE to α -Gal ≥ 0.35 IU/mL. In most cases allergists in practice reported using the α -Gal sIgE assay supported by Viracor (Lee's Summit, MO). Results of this assay, which is manufactured by Thermo-Fisher/Phadia and uses beef thyroglobulin on the solid-phase, have been shown to be highly correlated with the cetuximab-based α -Gal sIgE assay used in the research lab at UVA ($r^2 = 0.98$)²³.

Seroprevalence to Rickettsiales in cases and controls

ELISA assays to assess IgG serology to spotted fever group Rickettsia (SFG) were carried out according to the manufacturer's instructions (Fuller labs, Fullerton, CA) using sera diluted 1:64. Positive titers were determined using the internal calibrator. IgG antibodies to *Ehrlichia chaffeensis* were identified by a micro-immunofluorescence assay (Fuller labs) and examined using a fluorescent microscope (EVOS FL Auto, Life Technologies Inc.) according to the manufacturer's instructions. IgG to *E. chaffeensis* was determined by comparison to positive and negative control sera by two of the authors who reviewed the slides in a blinded fashion.

Mapping

Maps were generated using ArcMap software version 10.6 (Esri Inc., Redlands, CA, USA) based on the geographic coordinate information and the number of α -Gal and fire ant anaphylaxis cases reported by individual clinics. The quarantine areas for the IFA were established by the USDA (<https://www.aphis.usda.gov/aphis/maps/plant-health/ifa-quarantine-mapping>) and were used to generate the fire ant quarantine map. The distribution of *A. americanum* was approximated based on CDC maps (www.cdc.gov/ticks/maps/lone_star_tick.html)⁵.

Statistics

Normally distributed data were compared with Student's T-test and expressed as medians. IgE levels were compared with the Mann-Whitney U test and expressed as geometric means with 95% confidence intervals. Categorical variables were compared with Fisher's exact test and presence or absence of a given exposure was related to α -Gal sensitization status by calculations of odds ratios (GraphPad Prism, v7.0). The relationship between α -Gal sensitization

198 and demographic and environmental variables (age, sex, history of tick bite(s), history of
199 pruritic reactions to tick bite(s), cat ownership, dog ownership, farm animal exposure and
200 history of worm infections) was assessed with multiple variable logistic regression (IBM SPSS,
201 v26).
202

RESULTS

The epidemiology of α -Gal syndrome in the United States

The first phase of the survey was carried out beginning in 2016. Based on prior reports, we initially focused on the Southeast, and confirmed that most of the clinics surveyed in this area had diagnosed 5 or more cases of delayed anaphylaxis to mammalian meat in the past 3 years. The survey was then extended outward and in total we collected information from 152 clinics in 44 states and one Canadian province. Over 90% of allergists who were approached agreed to participate in the survey.

At least one provider in each of 11 states reported greater than 100 α -Gal cases. These states included Arkansas, Virginia, North Carolina, Tennessee and Missouri, as previously reported, but also Alabama, Kentucky, Georgia, Maryland, Oklahoma and New York (**Fig 1 and Table E1**)⁵. Of the 29 providers who reported greater than 40 cases, 28 were in the area where *A. americanum* was reported to be present in 2007, and none were west of the 100th meridian. Of note, many of the clinics close to the Gulf Coast had seen few or no cases despite being within the reported range of *A. americanum*^{24, 25}. For example, of 10 practices surveyed in the eastern half of Texas, none reported more than 5 cases and 6 reported no α -Gal cases at all. Another unexpected finding during our survey was that three separate providers in Northern Minnesota reported at least 5 cases, with one seeing over 40 cases consistent with α -Gal. This cluster of cases is in an area where *A. americanum* is not included in the CDC map of the tick territory, though there have been occasional reports according to the Minnesota Department of Health (<https://www.health.state.mn.us/>) and a recent citizen science initiative²⁶.

Of 59 providers who provided information about the effectiveness of dietary avoidance on allergic symptoms, 49 (83%) reported that >90% of their patients became free from allergic attacks by avoiding mammalian meat. An additional 15% of providers reported that at least 70% of their patients responded to mammalian meat avoidance, with some of these providers mentioning that dairy avoidance was additionally required for a minority of their patients.

Evidence that α -Gal has similar immunologic features in geographically distinct areas

To confirm that the syndrome has similar immunologic features in geographically distinct areas we conducted an analysis of 211 α -Gal patients from central Virginia (who were part of a recently reported cohort study) as well as 75 patients from an area including the corner of Missouri, Arkansas, and Oklahoma that we have called “Three Corners”¹⁹. The decision to study this area stemmed from multiple factors. The realization that α -Gal is common in this region dates back to the initial description of the syndrome and current surveys confirmed the relevance of α -Gal in this area. Our investigation included determination of α -Gal sIgE levels, total IgE levels and IgE to a panel of other common allergens, with all testing conducted at the UVA research lab. The median age was 50 years, males and females were similarly represented, and total IgE levels were similar in both groups (**Table E2**). Interestingly, the levels of α -Gal sIgE were higher in the subjects in Virginia than Three Corners (GM, 14.6 IU/mL vs 7.5 IU/mL, $p < 0.001$); nonetheless, the levels of α -Gal sIgE relative to total IgE exceeded 5% in the majority of subjects and the IgE assays to beef and pork were positive in >85% of both groups (**Fig 2A**). Moreover, levels of IgE to beef, pork and milk were also similar in the two groups (**Fig 2B**). IgE sensitization to two representative aeroallergens (dust mite and Timothy grass) and non-mammalian foods (peanut and wheat) was present in only a minority of subjects and had similar prevalence in both groups (**Table E1**). Notably, there was a difference in the frequency of IgE to venomous insects in the two populations with greater prevalence in Virginia than Three Corners (Honey Bee, 24% vs 9%, $p = 0.007$; Wasp, 38% vs 12%, $p < 0.001$).

Further evidence for a connection with tick bites

To investigate the relationship of tick bites and other exposures with α -Gal sensitization, we compared questionnaire data from 311 subjects from Virginia who were sensitized to α -Gal with 97 subjects who lacked sensitization (cut-off 0.35 IU/mL). While 97% of sensitized subjects reported histories of tick or ‘chigger’ bites, less than 10% reported histories of tick-borne infections. We included chigger bites in the survey because in our experience there are many patients who confuse bites from larval seed ticks with those from true chiggers, which are members of the Trombiculidae family of mites. In addition, there is some evidence suggesting that Trombiculidae chiggers may be able to induce sensitization to α -Gal²⁷. Exposures that were more common in sensitized than non-sensitized subjects included tick (or chigger) bites, history

of pruritic rash relating to tick bite, having an indoor/outdoor dog and having exposure to farm animals (**Table I**). Notably, very few subjects reported histories of worm infections. A history of tick bite(s) within the last 10 years had an odds ratio of 14.1 (95%CI 7-29) and a history of pruritus related to a tick bite had an odds ratio of 8.8 (95%CI 5-15) with α -Gal sensitization. In multiple variable logistic regression modeling, male sex, history of tick bites and a history of pruritic rash related to tick bites remained significant (**Table E3**).

To address the possibility that exposure to house cats could also be a relevant factor in α -Gal sensitization, as has been suggested by some reports, we investigated a cohort from an area where ticks are very rare^{28, 29}. Detailed information about cat exposure, as well as IgG to Fel d 1 as a marker of exposure, was available from a population-based study in Northern Sweden that we have previously investigated for asthma²⁰. In this cold and arid region that straddles the Arctic Circle ticks are rare and there has been little report of mammalian meat allergy^{30, 31}. Of the 90 subjects who reported cat ownership none had IgE to α -Gal (**Table E4**), and of the cohort of 413 that was investigated only a single subject (0.2%) had IgE to α -Gal $\geq$ 0.35 IU/mL.

Serology to tick-borne infections in subjects with α -Gal

While it is clear that most subjects with α -Gal syndrome do not report histories of tick-related febrile illnesses, we nonetheless considered the possibility that exposure to microbial symbionts present in tick saliva could be a co-factor relevant to α -Gal sensitization. To address this hypothesis we carried out assays to detect IgG antibodies to bacteria that have been frequently identified in *A. americanum* - Spotted fever group Rickettsia (SFG) and *E. chaffeensis*³². Of note, SFG includes not only *Rickettsia rickettsii*, the causal agent of RMSF, but also other species including *Rickettsia amblyommatis*. While *R. amblyommatis* is not known to be pathogenic to humans it is identified in a large proportion of both adult and larval lone star ticks³²⁻³⁴ and has been postulated to contribute to the high seroprevalence to SFG in the southeastern U.S.³⁵. We found that IgG antibodies to SFG antigen were relatively common in subjects from both central Virginia and Three Corners (ie, 21 and 43%) but the results were not significantly different than in control subjects from the same area who were not sensitized to α -Gal (**Table II**). Specificity of the assay was supported by the relatively low number of positive

sera from a comparison cohort where tick bites and α -Gal sensitization are uncommon, i.e. - Northern Sweden. IgG to *E. chaffeensis* was detected rarely in any of the populations we investigated (**Table II**).

Relevance of the imported fire ant (IFA) to explain the low prevalence of α -Gal cases on the Gulf Coast

Although studies on the role of fire ants as a predator of ticks were published as early as the 1970's we first became aware of a possible role of IFA in Texas during discussions at the 2017 Tick Summit in Maryland^{13, 14}. To assess the relationship between α -Gal, *A. americanum* and IFA we focused on the southeastern USA. Cases of α -Gal were overlaid on a map demonstrating regions of fire ant quarantine, stratified by the time of quarantine origination (**Fig 3**). This map demonstrated that α -Gal cases were much more common in areas that had never been quarantined, or had been quarantined relatively recently, as compared to areas where the IFA had been long established, i.e. – prior to 1975. Moreover, because fire ant anaphylaxis is in the purview of allergists we were able to re-visit the survey in clinics in the relevant areas with additional questions about fire ant cases. The results were consistent with an inverse relationship between IFA cases and α -Gal cases (**Fig 3**). Moreover, the map of fire ant anaphylaxis cases reported in the survey aligned closely with the fire ant quarantine map. (**Fig 4**). To further probe the relationship between α -Gal and fire ant we did an analysis of all clinics within the reported 2007 lone star distribution where we had survey data on both α -Gal and fire ant cases. Of the 70 clinics that met this criteria, 22 were in a region that had never been under fire ant quarantine, 17 were in a region that had been quarantined in 1975 or later, and 31 were in a region that had been under fire ant quarantine prior to 1975. The data clearly show an inverse relationship between α -Gal cases and fire ant cases in the area that has never been quarantined as compared to the area that had been quarantined prior to 1975 (**Fig 5A**). Importantly, sera from α -Gal and fire ant cases in one such clinic in Birmingham, Alabama and α -Gal cases in Three Corners confirms that the patients have distinct IgE serology (**Fig 5B**).

DISCUSSION

There are several major issues that we have addressed in the current report: firstly, information about the epidemiology of the α -Gal syndrome in the USA, including additional evidence that the immunologic features of the syndrome are similar in geographically distinct areas; secondly, further evidence for the connection with the lone star tick; thirdly, a novel explanation for an apparent “shortage” of α -Gal cases in the Deep South; finally, increasing evidence that fire ants represent another important and expanding regional cause of anaphylaxis. To date there has been little research on the epidemiology of the α -Gal syndrome. This, in large part, reflects the fact that the syndrome has only been recognized for 10 years, and that there is not a dedicated ICD diagnostic code. In general, the prevalence of α -Gal cases conformed well to the map of *A. americanum*, and the northern and western extent of cases appears to be largely determined by areas where bites from the lone star ticks are common. The explanation for the cases in northern Minnesota is not clear. There have been limited reports of *A. americanum* in these areas^{26, 36}, but an alternative explanation is that other North American ticks could also contribute to α -Gal sensitization. Different species of ticks cause α -Gal sensitization outside of North America, e.g., *Ixodes ricinus* in Europe and *Ixodes holocyclus* in Australia, and it has been recently reported that *Ixodes scapularis* (the black-legged tick) can express α -Gal epitopes^{37, 38}. Because *I. scapularis* and *Dermacentor variabilis* are present in northern Minnesota it is possible that these ticks could be relevant to α -Gal sensitization. On the other hand, the scarcity of α -Gal cases in other areas where these ticks are common, including the northeastern USA in the case of *I. scapularis*, suggests that these species of tick are not a major cause of sensitization³⁹. The paucity of α -Gal cases in the Deep South could have reflected a lack of awareness of the syndrome, though this was not true for the providers we spoke with. An alternative hypothesis is that *A. americanum* is no longer a dominant tick in this area. Because very few states have active tick surveillance programs this is not straightforward to determine; nonetheless, a recent citizen science initiative supports the idea that the lone star tick is not currently abundant in the Deep South²⁶. Our data are consistent with the view that the decrease in ticks relates to predation or other competitive pressures exerted by fire ants. We would highlight that many of the studies which described a predatory role for IFA were

published in the 1970's^{13,14}. More recent work raises the possibility that tick populations are impacted by IFA via indirect mechanisms, e.g. – by altering tick behavior or reducing small mammal populations^{15,16}. There are predictions that climate change could impact the range of ticks in Texas and the Gulf Coast, but it is unclear that this has had a major influence yet²⁴.

Although the α -Gal syndrome has been recognized in wide areas of the USA and other parts of the world, there have been few head-to-head comparisons of patients from different geographic areas. Here we show that patients from “Three Corners” and central Virginia had a very similar pattern of IgE responses to α -Gal and mammalian extracts, with the notable caveat that levels of IgE to α -Gal were lower in Three Corners than Central Virginia. The explanation for this is unclear but suggests that there could be subtle differences in the factors that contribute to sensitization and/or in the patient populations that were investigated. An interesting possibility, hinted at by the greater prevalence of sensitization to insect venom in central Virginia, is that insect stings could promote enhanced IgE responses to α -Gal and/or tick bites. At this point we are not in doubt that the sensitization to α -Gal is a consequence of tick bites because of: (i) the cumulative evidence published from Virginia^{5,7,40} (ii) the correlation with bites from other ticks in Australia, Japan, South Africa and Sweden⁸⁻¹¹, (iii) evidence that ticks carry α -Gal^{38,41-44}, and (iv) emerging evidence from mouse models^{42,45}. Accepting that the α -Gal antigen must be present in tick saliva at the time of a blood feed, there are multiple possible sources for the carbohydrate. Glycoproteins carrying α -Gal could be present in the saliva as part of the regurgitated blood meal from a recent mammalian host, α -Gal could be synthesized by microbial symbionts (such as Rickettsia or Ehrlichia) present in the tick saliva, or the α -Gal could be directly produced by the tick salivary glands. The data presented here is in keeping with the view that symbionts can induce an immune response to Rickettsial antigens without a clinically evident febrile illness, nonetheless our evidence does not show a correlation between an IgG response to these bacteria and IgE to α -Gal (**Fig E3**). Collectively, current evidence is most consistent with the view that α -Gal is intrinsically produced in the saliva of many species of ticks, including *A. americanum*, during a bloodfeed^{38,44}.

For over 40 years the IFA has been appreciated as an important regional cause of allergic reactions, including cases of fatal anaphylaxis⁴⁶⁻⁴⁸. Nonetheless, most reports on IFA

anaphylaxis have been restricted to cohort studies at single institutions or practices in the Southeast. The current survey supports the view that allergic reactions to IFA are common in a large area of the country where the colonies are established. In coming decades it is anticipated that allergic reactions to IFA will be increasingly recognized in areas of the Mid-Atlantic and Midwest as a result of ongoing climate change⁴⁹. Importantly, it is very unlikely that providers would confuse reactions caused by fire ants with the reactions attributable to the α -Gal syndrome. Both can culminate in systemic anaphylaxis, but the intense pain and cutaneous pustules which result from a fire ant sting are readily recognizable⁵⁰. The question of whether there could be a glycan(s) in fire ant venom which could be a relevant allergen in IFA anaphylaxis is an interesting question. Hoffman and colleagues reported that the major IFA allergen Sol i 1 carries a mannosylated N-glycan(s) which is cross-reactive with a carbohydrate present in honey bee venom⁵¹. However, α -Gal is structurally distinct from the cross-reactive carbohydrate determinants (CCD) of fire ants and bee venom⁵². The fact that α -Gal is not a feature of IFA venom is strongly supported by the inverse relation between α -Gal and IFA cases, and also the distinct serologic profile of subjects reporting fire ant anaphylaxis and α -Gal syndrome.

There are several limitations to our results. Carrying out a disease survey before there is an ICD-9 or 10 code is difficult, and the modified snowball approach is not designed to determine prevalence or incidence rates in the population. The approach we used would not have been useful before the condition was well recognized or before there was an assay for IgE antibodies to α -Gal available. The initial design of the survey occurred in 2015 and was carried out in 2016-2017, by which time all the allergists contacted were aware of the condition and had either identified cases or investigated possible cases. We also believe that the telephone format provided more reliable results than a questionnaire mailed to allergists where the response rate is generally very low. Case numbers provided by physicians reflected estimates and could certainly be subject to recall bias. Some cases were diagnosed and/or acquired when patients were in a different part of the country. Despite these limitations we would highlight that our data match well with an online survey of individuals self-reporting mammalian meat allergy (<https://www.zeemaps.com/map?group=555038>). Although several of the allergists who

were surveyed are known to the authors, the snowball approach minimized this bias by facilitating the participation of providers unknown to the authors. In the comparison between Virginia and Three Corners we did not have case-specific information about allergic history for subjects from Three Corners. Nonetheless, in conversations with providers from those clinics the syndrome presents similarly to our experience in Virginia¹⁹, i.e. - urticaria, gastrointestinal distress and/or anaphylaxis which is often delayed and responds to a diet avoiding mammalian meat. We fully recognize that we had no awareness of the possible interaction between fire ants and ticks until after we had completed most of the initial survey. As a consequence, not all providers who participated in the survey provided information on IFA cases. We are grateful to several participants of the Tick Summit meeting in Feb 2017 who encouraged us to investigate the relevance of fire ants as predators of ticks. We also recognize that the analysis of a possible role of symbionts is incomplete. Such an analysis is dependent on knowledge of these bacteria and the availability of serum assays, however *R. amblyommatis* and *E. chaffeensis* are considered to be the dominant symbionts of lone star ticks in the area where the α -Gal syndrome is common³².

Conclusions

The results presented here reveal that cases of the α -Gal syndrome are now common in a large area of the United States including significant parts of at least 14 states, and also that fire ant anaphylaxis is a major problem in many areas of the Gulf Coast and Texas. The evidence overall suggests that the α -Gal syndrome relates to bites from lone star ticks or their larvae, although it is possible that other North American ticks could also contribute to some of the cases. It remains most likely that saliva from certain species of ticks contains α -Gal glycans which, together with other factors, promote IgE class-switch and Th2-immunity. Thus, tick saliva introduced via the skin induces the IgE response to this blood-group-like antigen which is the target of pre-existing natural antibodies in healthy humans^{6, 7}. The evidence also shows a negative correlation between α -Gal cases and established colonies of the IFA. This is a dynamic situation where the upper range of the lone star tick is moving north⁵³, but at the same time fire ants continue their relentless spread north. Expansion of the fire ant into new parts of the Southeast and Mid-Atlantic will likely control the population of lone star ticks in these areas and

430 lead to a decrease in cases of tick-related meat allergy. On the other hand, it can be expected
431 that IFA anaphylaxis will emerge as a new problem in these areas.

432 **ACKNOWLEDGMENTS:** Appreciation to Sean Morris and Kristin Wells for helpful discussions
433 about geospatial mapping. We are grateful to all of the allergists who participated in the survey
434 and contributed to data for the mapping.

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TABLES

Table I. Demographics and exposure history in subjects recruited from an allergy clinic in Central Virginia, stratified by α -Gal sIgE status.

Demographic or Exposure	α -Gal negative (<0.35 IU/mL)	α -Gal positive (≥ 0.35 IU/mL)	OR (95% CI)	P value
n	97	311	na	na
Age at enrollment, median (range)	47 (12-78)	50 (5-77)	na	na
Sex, male	27 (28%)	145 (47%)	2.3 (1.4-3.7)	0.001
History of tick or chigger bites	66 (68%)	301 (97%)	14.1 (6.8-28.6)	<0.001
lone star in last 10yrs	21 (22%)	160 (51%)	3.8 (2.1-6.0)	<0.001
deer ticks in last 10yrs	39 (40%)	199 (64%)	2.6 (1.6-4.2)	<0.001
dog ticks in last 10yrs	27 (28%)	109 (35%)	1.4 (0.9-2.3)	0.22
seed ticks in last 10yr*	21 (22%)	184 (59%)	5.2 (3.1-9.0)	<0.001
chiggers in last 10yrs*	34 (35%)	193 (62%)	3.0 (1.9-4.8)	<0.001
History of pruritic rash related to tick bite	45 (46%)	275 (88%)	8.8 (5.2-15.0)	<0.001
duration of months or longer	2 (2%)	53 (17%)	9.8 (2.6-41)	<0.001
History of tick-borne infection	7 (8%)	30 (9%)	1.4 (0.6-3.4)	0.55
Lyme Disease	6 (6%)	19 (6%)	1.0 (0.3-2.1)	0.98
RMSF	0	4 (1.3%)	∞ (0.3- ∞)	0.26
Ehrlichiosis	0	2 (0.6%)	∞ (0.1- ∞)	0.43
Have an indoor cat	33 (34%)	136 (44%)	1.5 (0.9-2.4)	0.10
Have an indoor dog	46 (47%)	187 (60%)	1.7 (1.1-2.6)	0.03
Have contact with farm animals	17 (18%)	106 (34%)	2.4 (1.4-4.0)	0.002
History of worm infection	3 (3%)	16 (5%)	1.7 (0.5-5.6)	0.6

*'Seed ticks' and 'chiggers' are widely used in Virginia to describe larval lone star ticks, however chiggers can also refer to mites of the Trombiculidae family²⁷.

589 **Table II.** Assessment of IgG antibodies to Rickettsia spotted-fever group (SFG) and *E. chaffeensis* and
 590 their relationship to α -Gal sensitization in subjects from areas where ticks are common (Central Virginia
 591 and Three Corners) or rare (Northern Sweden).

Characteristics	Central Virginia		Three Corners		Northern Sweden	
	α -Gal neg	α -Gal pos	α -Gal neg	α -Gal pos	α -Gal neg	α -Gal pos
n assayed for Rickettsia SFG	89	280	29	75	100	0
Rickettsia SFG IgG positive, n (%)	12 (13.5%)	59 (21.1%)	8 (27.6%)	32 (42.7%)	6 (6%)	-
n assayed for Ehrlichia	30	90	29	78	nd	nd
<i>E. chaffeensis</i> IgG positive, n (%)	0 (0%)	3 (3.3%)	1 (3.4%)	10 (12.8%)	nd	nd

592 nd – not done

FIGURE LEGENDS

Fig 1. Cases of α -Gal syndrome identified from a survey of allergists in practice in the United States, overlaid with CDC-reported distribution of *Amblyomma americanum* (approximated), often called the lone star tick. The territory of the tick in 2011 included the area that was described in the 2007 CDC map.

‡ These cases had all been diagnosed elsewhere.

Fig 2. (A) Specific IgE to α -Gal in relation to total IgE among subjects presenting with mammalian meat allergy in Virginia and Three Corners, and also control subjects from Virginia without history of reactions to mammalian meat. **(B)** Specific IgE to α -Gal and mammalian extracts in subjects presenting with mammalian meat allergy in Virginia and Three Corners.

Fig 3. Map highlighting a region of the USA where many α -Gal cases were identified and *A. americanum* is reported to be established by the CDC. Cases of α -Gal syndrome and fire ant anaphylaxis are overlaid with county-level fire ant quarantine data, categorized by date of the initial quarantine (IFA quarantine data courtesy of the USDA - www.aphis.usda.gov/aphis/maps/plant-health/ifa-quarantine-mapping). Each sequential quarantine zone also includes the territory in the earlier quarantine zone.

Fig 4. Cases of fire ant anaphylaxis identified in a survey of allergists in the United States

focused in areas where the lone star tick is endemic and/or fire ants have been reported. IFA

cases were overlaid with county-level fire ant quarantine data, categorized by date of the initial

quarantine. Each sequential quarantine zone also includes the territory in the earlier quarantine

zones.

‡ Each of these three areas has local reports of IFA infestation

Fig 5. (A) Cases of α -Gal syndrome and fire ant anaphylaxis among clinics within the established

range of the lone star tick. The analysis was restricted to clinics which reported data on both α -

Gal and fire ants. Cases of α -Gal and fire ant were compared to each other by Mann-Whitney U

test within each area. Also, cases of fire ant anaphylaxis were compared between areas and

were fewer in the never quarantine area as compared to both areas that have been

quarantined, $p < 0.001$ (#). Similarly, α -Gal cases were compared between areas and were fewer

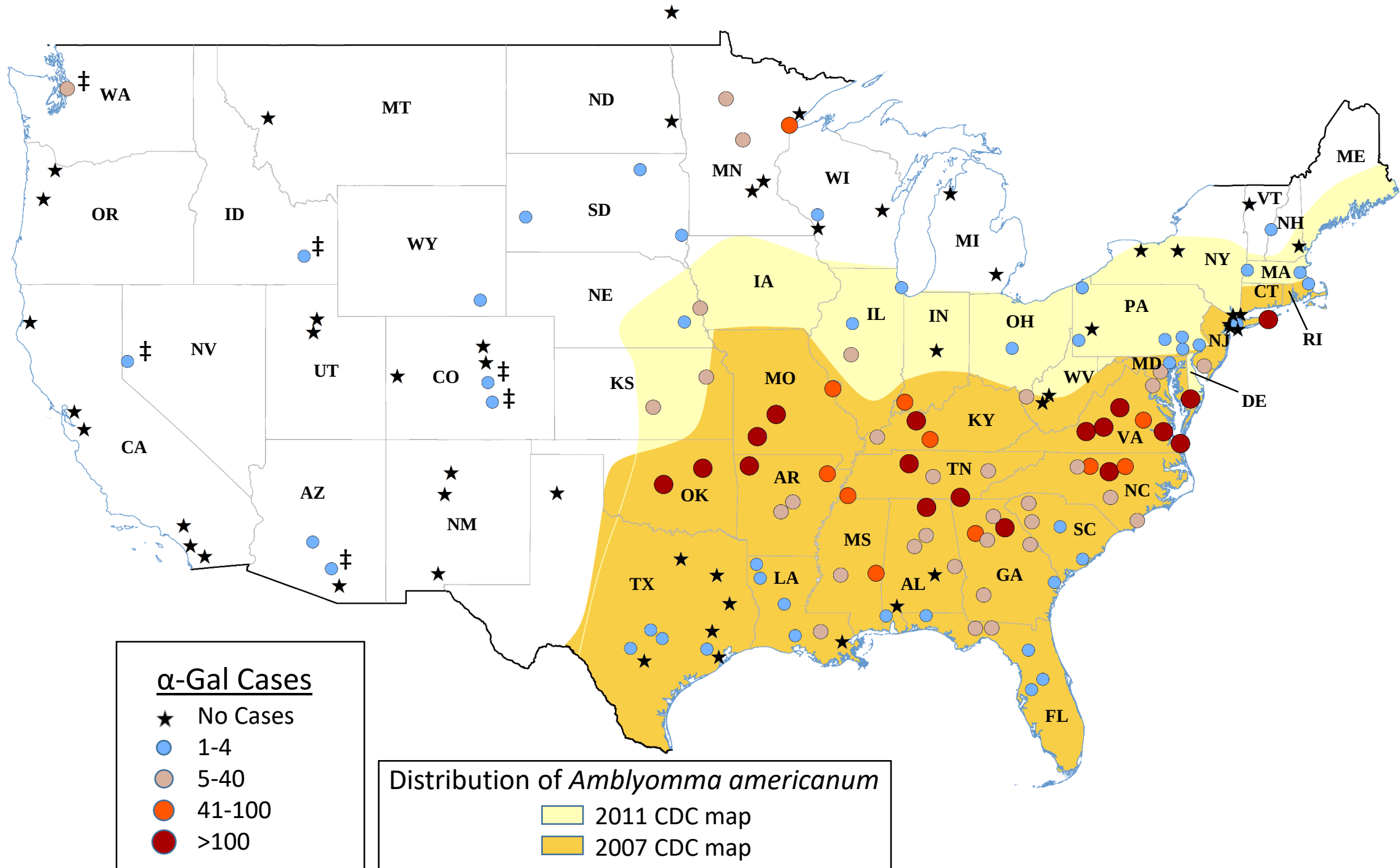
in the area that had been quarantined for fire ants prior to 1975 as compared to the area

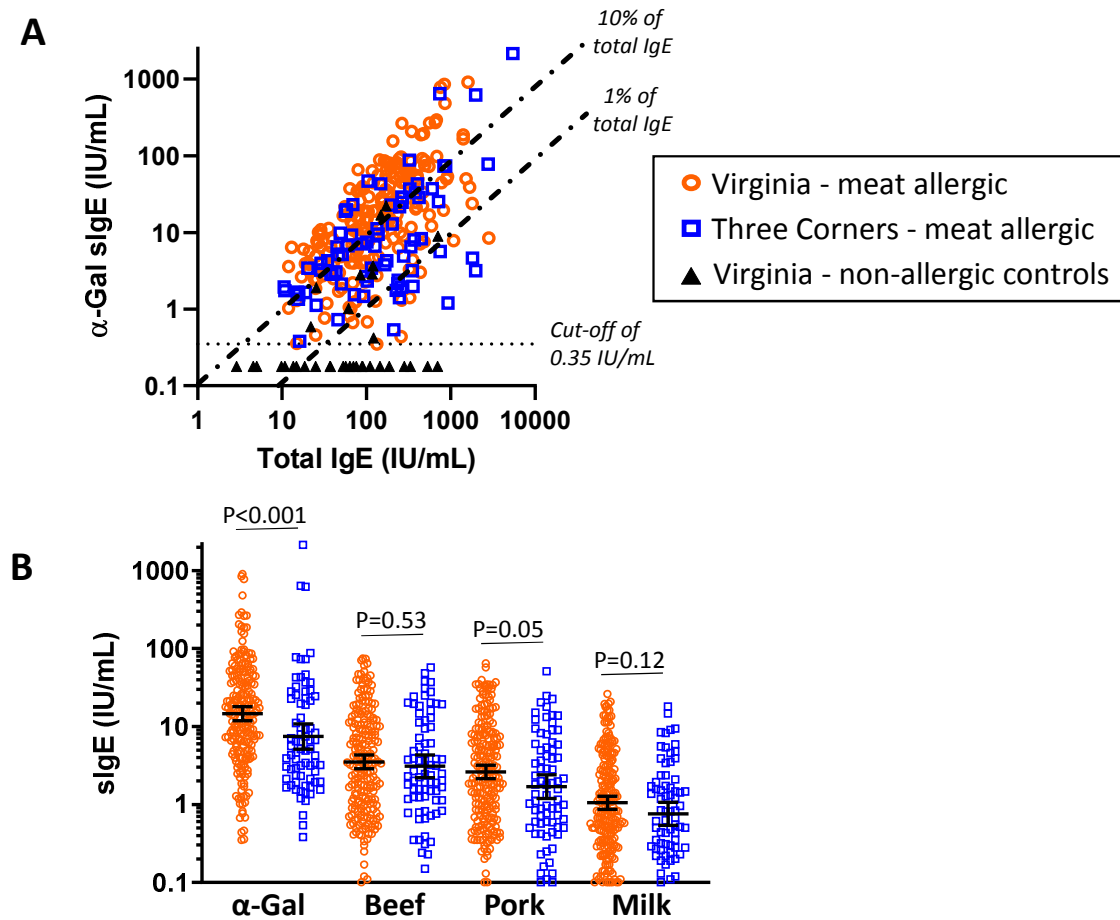
quarantined from 1975 to 2018 or the area that has never been quarantined, $p < 0.001$ (##). **(B)**

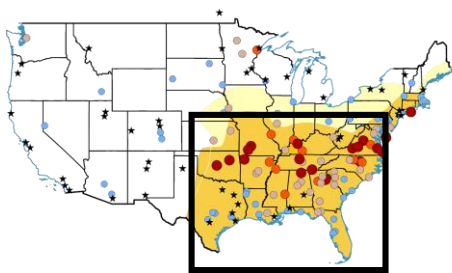
Plot of IgE to fire ant and IgE to α -Gal among cases evaluated in 'Three Corners' and

Birmingham, AL for mammalian meat allergy ($n=75$ in Three Corners and $n=8$ in Birmingham)

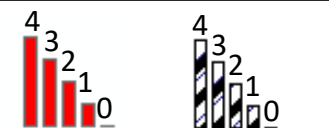
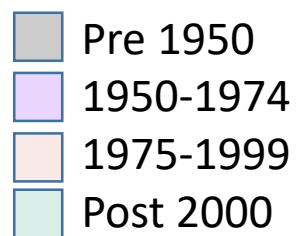
and fire ant anaphylaxis ($n=0$ in Three Corners and $n=24$ in Birmingham).





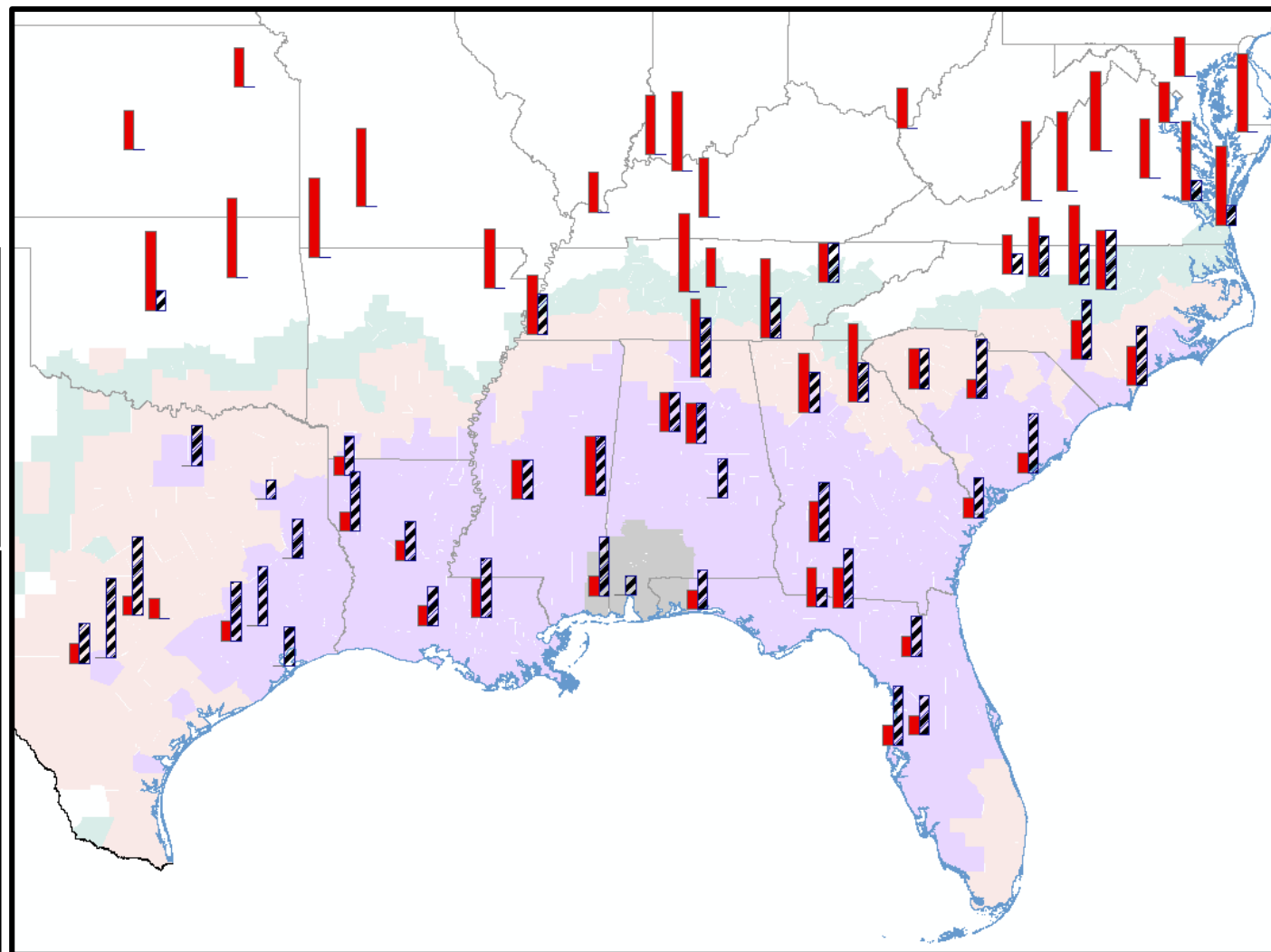


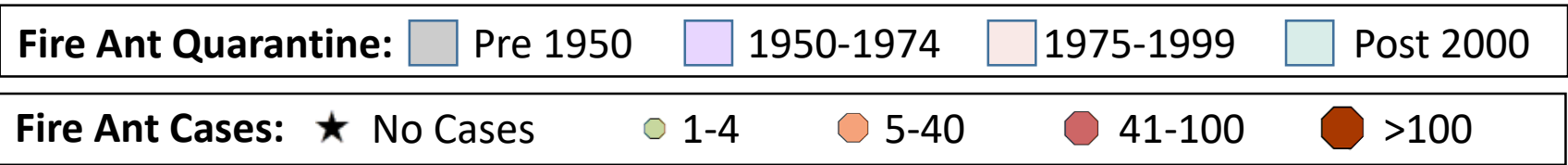
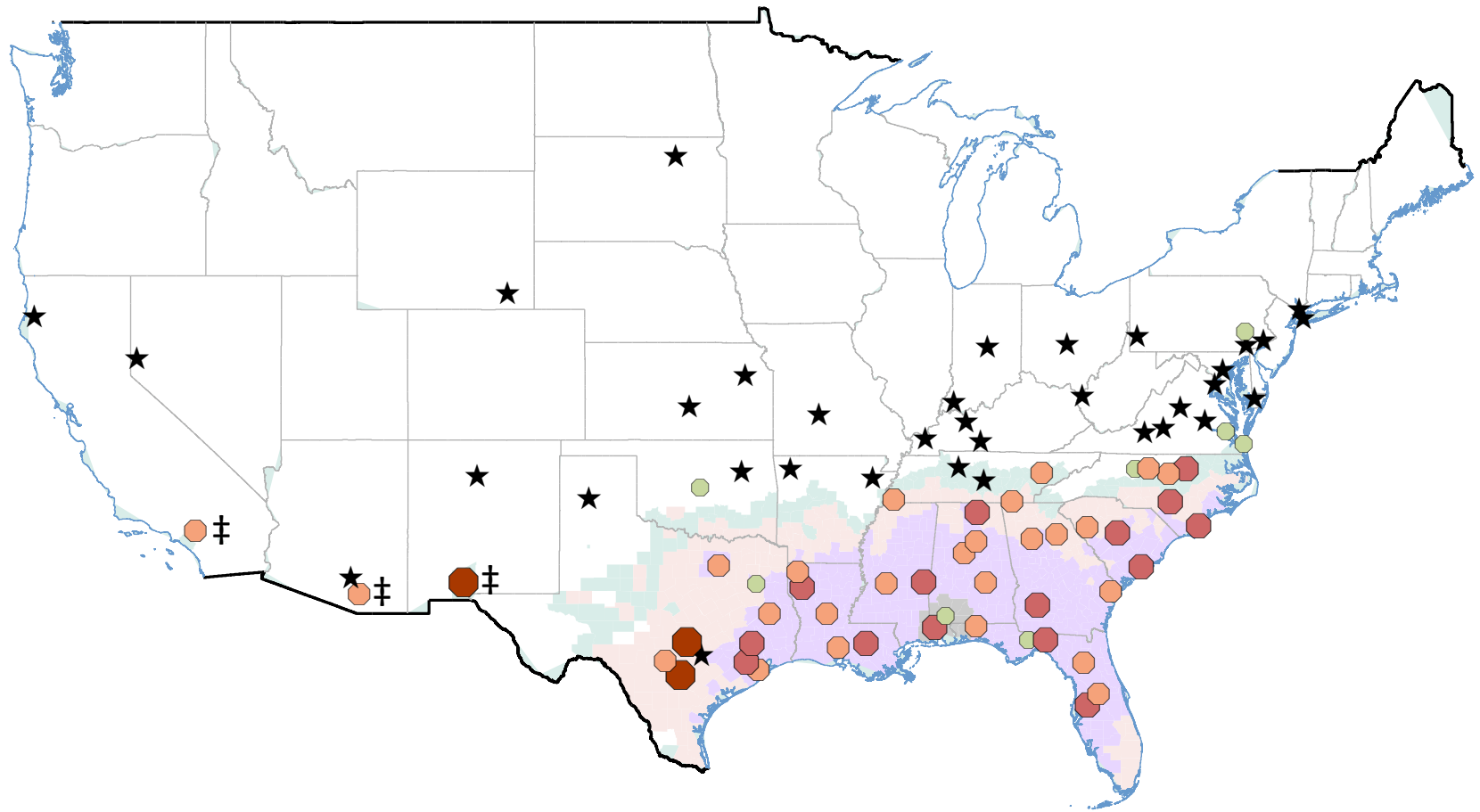
Fire Ant Quarantine



α -Gal **Fire ant**

4: >100 cases
 3: 41-100 cases
 2: 5-40 cases
 1: 1-4 cases
 0: no cases

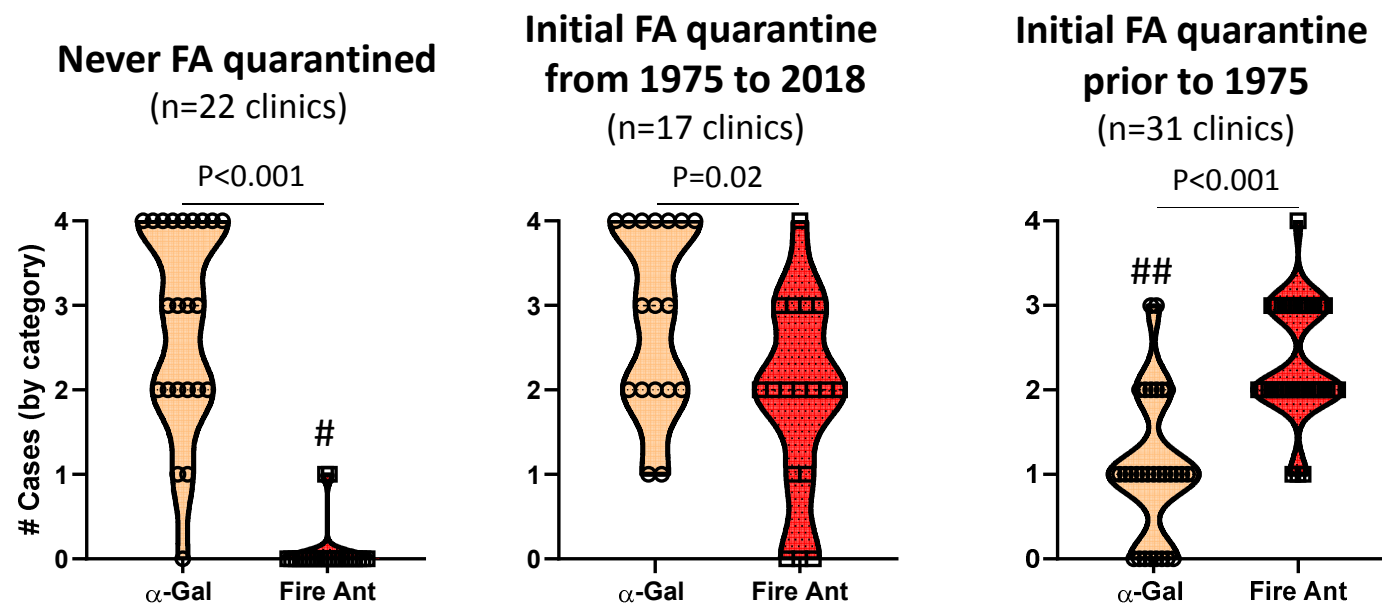




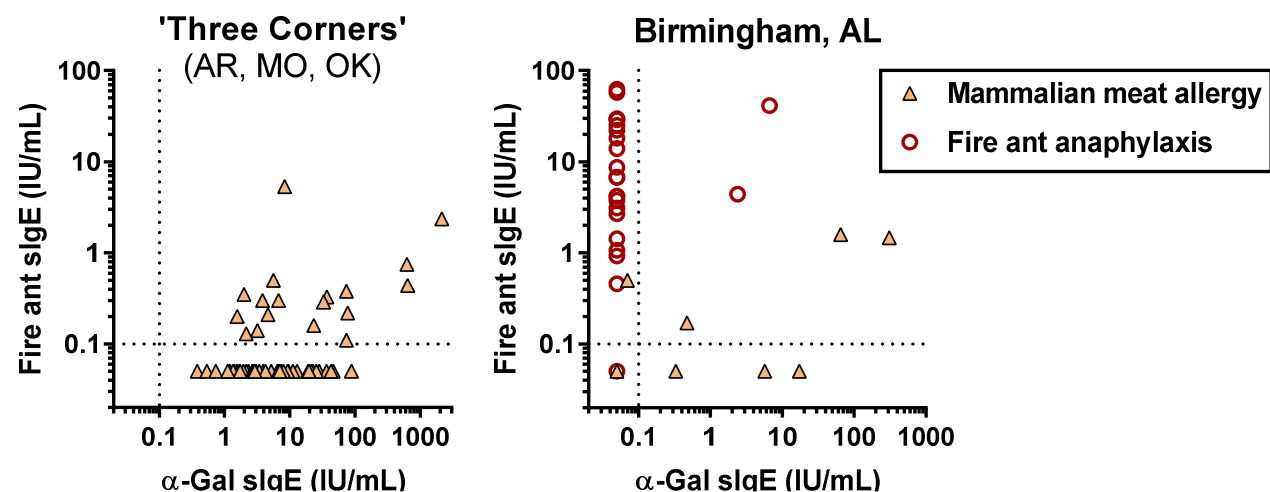
A

**Code for # of α -Gal
and Fire ant cases**

0: None
1: 1-4
2: 5-40
3: 41-100
4: >100



B



Name of Physician _____ Office Contact _____
 Town/State _____ Date _____
 Telephone (1) _____ (2) _____
 Email (or other contact) _____

Delayed Anaphylaxis to Red Meat Questionnaire

Are you aware of the basic details of delayed anaphylaxis to red meat? Yes _____ No _____

How many have you tested on the basis of history? _____; or

0 1-4 5-40 40-100 >100

Have you diagnosed cases in your practice? Yes _____ No _____

If so, how many in the last 3 years? # _____ or

0 1-4 5-40 40-100 >100

Are you aware of any other practices in your region or state that are seeing cases? _____;

- contact information for those practices _____

If you have seen cases of delayed anaphylaxis to red meat:

a) How did you confirm cases: Skin tests _____ Laboratory tests _____
 Approximately how many of the cases you tested because of a suggestive history have been negative for IgE to alpha-gal? _____

b) What is the range of times before start of reactions after eating mammalian products?

c) Do you question patients about: Tick bites _____ Insect bites _____ Chiggers _____

d) How common is a positive answer about tick or chigger bites? < 50% 50-70% >70%

In confirmed cases:

What % become free of allergic attacks on a diet avoiding red meat? <50% 50- 70% 70-90% >90%

If symptoms persist, do you recommend: Dairy Free diet _____ or other treatment _____

In the area your patients come from, how common is it for deer to come close to houses?

Never Occasional Regular

Name of Physician _____ Office Contact _____
 Town/State _____ Date _____
 Telephone (1) _____ (2) _____
 Email (or other contact) _____

Delayed Anaphylaxis to Red Meat Questionnaire

Are you aware of the basic details of delayed anaphylaxis to red meat? Yes _____ No _____

How many have you tested on the basis of history? _____; or

0 1-4 5-40 40-100 >100

Have you diagnosed cases in your practice? Yes _____ No _____

If so, how many in the last 3 years? # _____ or

0 1-4 5-40 40-100 >100

Are you aware of any other practices in your region or state that are seeing cases? _____;

contact information for those practices _____

If you have seen cases of delayed anaphylaxis to red meat:

a) How did you confirm cases: Skin tests _____ Laboratory tests _____

Approximately how many of the cases you tested because of a suggestive history have been negative for IgE to alpha-gal? _____

b) What is the range of times before start of reactions after eating mammalian products? _____

c) Do you question patients about: Tick bites _____ Insect bites _____ Chiggers _____

d) How common is a positive answer about tick or chigger bites? < 50% 50-70% >70%

In confirmed cases:

What % become free of attacks on a diet avoiding red meat? <50% 50- 70% 70-90% >90%

If symptoms persist, do you recommend: Dairy Free diet _____ or other treatment _____

Experience IFA allergy:

How many cases have you seen that relate to allergic reactions to fire ant bites in the last 3 years?

0 1-4 5-40 40-100 >100

How many on ImmunoTherapy (IT) for Fire Ant? _____

Are you aware of any other practices in your region or state that are seeing cases? _____;

contact information for those practices _____

