The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-α-1,3-galactose

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Background: In 2009, we reported a novel form of delayed anaphylaxis to red meat that is related to serum IgE antibodies to the oligosaccharide galactose-α-1,3-galactose (alpha-gal). Most of these patients had tolerated meat for many years previously. The implication is that some exposure in adult life had stimulated the production of these IgE antibodies.
Objectives: We sought to investigate possible causes of this IgE antibody response, focusing on evidence related to tick bites, which are common in the region where these reactions occur.
Methods: Serum assays were carried out with biotinylated proteins and extracts bound to a streptavidin ImmunoCAP.
Results: Prospective studies on IgE antibodies in 3 subjects after tick bites showed an increase in levels of IgE to alpha-gal of 20-fold or greater. Other evidence included (1) a strong correlation between histories of tick bites and levels of IgE to alpha-gal ($\chi^2 = 26.8$, $P < .001$), (2) evidence that these IgE antibodies are common in areas where the tick *Amblyomma americanum* is common, and (3) a significant correlation between IgE antibodies to alpha-gal and IgE antibodies to proteins derived from *A americanum* ($r_s = 0.75$, $P < .001$).
Conclusion: The results presented here provide evidence that tick bites are a cause, possibly the only cause, of IgE specific for alpha-gal in this area of the United States. Both the number of subjects becoming sensitized and the titer of IgE antibodies to alpha-gal are striking. Here we report the first example of a response to an ectoparasite giving rise to an important form of food allergy. (J Allergy Clin Immunol 2011;127:1286-93.)

Key words: *Ticks, anaphylaxis, oligosaccharide, alpha-gal, IgE antibody to CCD*

The mAb cetuximab, which is specific for epidermal growth factor receptor, was approved for use in treating cancer in 2005.[1,2] Shortly thereafter, it became clear that a significant number of patients were experiencing severe hypersensitivity reactions during their first infusion of this mAb. Surprisingly, those reports appeared to be restricted to an area of the Southeast, including Tennessee, North Carolina, Arkansas, Virginia, and the southern half of Missouri.[3,4] In 2007, a study was published by the oncology groups at the University of North Carolina and Vanderbilt, suggesting that the prevalence of severe reactions to cetuximab was as high as 20%.[3] At that time, a detailed investigation of serum antibodies established that these reactions were occurring in patients who had preexisting IgE antibodies specific for the glycosylation on the Fab fragment of the mAb.[5] The relevant oligosaccharide is galactose-α-1,3-galactose (alpha-gal), which is a blood group substance of nonprimate mammals.[6,7] This analysis was, in part, made possible because Dr Zhou and his colleagues, working at ImClone (New York, NY), had published the full glycosylation of cetuximab.[8]

After establishing the assay for IgE antibodies to cetuximab (ie, alpha-gal), we screened large numbers of sera. The results established that these IgE antibodies were regionally distributed in a pattern consistent with the cases of cetuximab-related anaphylaxis. More importantly, the results led to the realization that IgE to alpha-gal was also associated with a novel form of food allergy.[5,9] Those patients reported that they had generalized urticaria or frank anaphylaxis starting 3 to 6 hours after eating beef, pork, or lamb; however, they reported tolerating chicken, turkey, or fish without difficulty.[9,10] Thus, the specificity of IgE antibodies present in their serum, which was known to be specific for a carbohydrate common to mammals but absent from poultry and fish, matched their symptoms.[6,9] In most cases, these patients were adults who had consumed red meat for many years before having the delayed reactions. This history implies that some new exposure had triggered the production of IgE antibodies to alpha-gal.

Initially, we investigated the possible role of a regionally important inhalant or fungal allergen, but those results were

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Abbreviation used
Alpha-gal: Galactose-α-1,3-galactose

uniformly negative. The next possibility was that a regional helminth could have induced IgE antibodies to the oligosaccharide. However, both from studying the known distribution of helminths in the United States and from sharing sera with Dr Amy Klion at the National Institutes of Health, we excluded *Ascaris* species and found little evidence for any other helminth.[11] By contrast, the known distribution of the immediate reactions to cetuximab was similar to the areas with high prevalence of Rocky Mountain spotted fever.[12] In addition, this area is similar to the area for maximum prevalence of human ehrlichiosis.[13,14] We were also aware of patients who thought that their reactions to red meat started after receiving multiple tick bites. Finally, we were informed that Dr van Nunen in Sydney, Australia, and Dr Deutsch in Georgia (personal communication, 2010) had reported to their local allergy meetings about patients who had become allergic to meat after experiencing multiple tick bites.[15] We report here the evidence that tick bites in the United States can induce IgE antibodies to alpha-gal. The evidence comes from (1) prospective studies of the response to tick bites in 3 subjects, (2) epidemiologic evidence that these IgE antibodies are present in areas where tick bites are common, (3) correlation between IgE antibodies to tick proteins and IgE antibodies to alpha-gal, and (4) evidence for an expanding range of the lone star tick, *Amblyomma americanum*.

METHODS

Patients, control subjects, and random populations

Clinic populations in Virginia. Patients presenting to clinic in Charlottesville for evaluation of recurrent anaphylaxis or severe urticarial reactions (n = 121), asthma (n = 56) or control subjects (n = 40) were enrolled and provided serum. Of these, the most recently enrolled 125 subjects responded to a full questionnaire, including questions about tick bites (see Fig E1 in this article's Online Repository at www.jacionline.org). Patients who specifically presented with urticarial or anaphylactic reactions were enrolled as subjects and had their serum tested for IgE antibodies to alpha-gal. Other patients were enrolled sequentially upon their agreeing to the informed consent process.

Random populations in the southeastern United States where tick bites are common. The Tennessee control cohort was obtained as a random control population in a cancer-screening clinic at Vanderbilt University.[5] The North Carolina cohort was collected randomly from hospital patients at the University of North Carolina as part of a study on the genetics of this anaphylactic response (unpublished data). The Virginia patients included 70 with acute asthma and 66 control subjects presenting to the emergency department at the University of Virginia.[16,17] In this last population there was no difference in the prevalence of IgE to alpha-gal between the patients with asthma and the control subjects.

Populations in areas where tick bites are rare. Subjects in the Boston Women's Study were enrolled as part of a birth cohort in a large urban center where tick bites are rare.[18] As part of a prospective study on asthma among children in the Norrbotten area of northern Sweden, sera were obtained from 963 subjects at age 18 years.[19] From these sera, all those with positive assay results for IgE antibodies to cat epithelium and dander of class 2 or higher (n = 150) were assayed for IgE to alpha-gal. Northern California was included because reactions to cetuximab are rare, and although some ticks are present there, *A americanum* is not.[13] The sera came from 3 cohorts: (1) patients with cancer at Stanford University,[5] (2) patients with acute asthma (n = 60) and control subjects (n = 57) presenting to Travis Air Force Base hospital near Sacramento,[20] and (3) patients with asthma (n = 102) and control subjects (n = 41) enrolled in studies on asthma at the University of California San Francisco.

Populations from Kenya and the Esmeraldas Province of Ecuador (ie, tropical areas where tick bites are common). In a previous study in Kenya, we reported that IgE antibodies to cat were common among schoolchildren in the village of Kabati (n = 131) and less common in the small town of Thika (n = 123). In the village, helminth infections, tick bites, and other ectoparasites are extremely common and less so in the town.[21] Sera from children in the Esmeraldas Province of northern Ecuador (n = 295) were collected as part of ongoing studies on the relevance of helminth infections to asthma and allergic disease.[22]

Approval for these studies was obtained locally in the area where subjects were enrolled and from the University of Virginia Human Investigation Committee. Further details regarding the individual cohorts can be found in Appendix E1 (available in this article's Online Repository at www.jacionline.org) and in the indicated references.

Allergen source and preparation

Adult, pathogen-free *A americanum* and *Dermacentor variabilis* ticks were purchased from the Oklahoma State Tick Rearing Facility (Stillwater, Okla) and were maintained at 4°C until preparation of whole-body extracts. The ticks were frozen with liquid nitrogen and crushed with a mortar and pestle. Proteins from the resultant powder were extracted overnight in borate-buffered saline, pH 8.0, with added protease inhibitors (Sigma Aldrich, St Louis, Mo). After removal of the solid pellet, the protein solution was defatted with ethyl ether (Sigma Aldrich), and after centrifugation at 3000 rpm for 5 minutes, the sample was separated into 3 layers, the bottom of which (the aqueous layer) was collected for analysis.[23,24]

ImmunoCAP IgE assays

Total and specific IgE antibodies were measured by using either commercially available ImmunoCAP (Phadia US, Portage, Mich) or a modification of the assay with streptavidin on the solid phase.[25] The assays were performed with the ImmunoCAP 250 instrument, and the results were expressed as international units per milliliter, with the international unit both for specific and total IgE being approximately 2.4 ng. For specific assays, the standard cutoff point for a positive reaction was 0.35 IU/mL. The streptavidin CAP technique was used to measure IgE antibodies to alpha-gal and tick proteins (*A americanum* and *D variabilis*), with approximately 5 μg of biotinylated antigen added to each CAP before adding 40 μL of undiluted serum. This assay is now available commercially. Selected sera were tested with commercially available assays for IgE antibodies to dust mite (*Dermatophagoides pteronyssinus*; d1), dog epithelium (e2), ryegrass pollen (g5), beef (f27), *Trichophyton rubrum* (m205), common silver birch pollen (t3), and German cockroach (i6).

Statistical analyses

We compared quantitative measures of IgE antibodies by using Spearman rank correlation and compared qualitative measures of tick bite severity and the presence of IgE antibodies by using the χ^2 test for trend. A 2-sided *P* value of less than .05 was considered to indicate statistical significance. Statistical analyses were performed with SPSS software, version 18.0 (SPSS, Inc, Chicago, Ill), and GraphPad Prism, version 4 (GraphPad Software, Inc, La Jolla, Calif).

RESULTS

Rapid development of IgE antibodies to α-gal after tick bites in 3 subjects

Over the last 3 years, we have had the opportunity to follow serum IgE responses prospectively in 3 subjects whose sera were available from before they experienced multiple tick bites. In each case IgE antibody levels to alpha-gal increased more than 20-fold after tick bites, and there was a parallel, although not identical,

increase in total IgE levels (Fig 1). Furthermore, 2 of these subjects (nos. 1 and 3 in Fig 1 and Table I) experienced an episode of generalized urticaria 3 to 4 hours after eating red meat, something that had not occurred before the tick bites. In these 2 cases the IgE antibodies to alpha-gal represented 30% or more of the total IgE. By contrast, in subject 2 IgE levels to α-gal increased from less than 0.35 to 8 IU/mL, but this accounted for less than 1% of the total IgE, and this subject has yet to report any allergic symptoms after eating mammalian meat. IgE antibodies to several other allergens were measured in serial samples from each of these subjects (Table I). The results show that none of the 3 subjects had new specificities of IgE antibodies, other than those that could be explained by IgE antibodies to alpha-gal (eg, cat, dog, beef, and milk). Although IgE antibodies to inhalants in sera from subject 2 did increase, these increases were much less than the increase in IgE levels to alpha-gal. In cases 1 and 2 the ticks responsible for the bites were identified as *A americanum*.

Relationship between histories of tick bites and serum IgE antibody levels to alpha-gal on a local and regional level

From questionnaire results, it was clear that a large proportion (>90%) of the subjects with serum IgE antibodies to alpha-gal had a history of tick bites. However, tick bites are now common in central Virginia. Among 125 subjects, including patients with delayed anaphylaxis, as well as patients with asthma and control subjects, a positive response to questions, including "Have you ever had local reactions to tick bites last for weeks or longer?" correlated highly significantly with the presence of IgE antibodies to alpha-gal ($P < .001$, Fig 2). We have now assayed sera or received results on more than 300 subjects who presented with anaphylaxis or urticaria and about whom we knew where they lived at the time of the first reaction. Although this is by no means a random sample, the results are interesting. Plotting positive cases on a map of the United States showed a striking similarity to the known distribution of *A americanum*.[13]

Although ticks are ubiquitous in temperate and tropical regions, there are major regional differences in species and also regions or populations where tick bites are rare or absent (Table II).[5,9,16-22] These include large cities and areas where the climate prevents survival of ticks or their natural hosts (eg, deer and small mammals). In keeping with the absence of ticks in the inland areas of northern Sweden, assays of sera from Norrbotten were negative for IgE antibodies to alpha-gal (Table II).[20] In contrast, we have assayed sera from Africa and Ecuador, which show a significant number of positive sera among rural populations. The positive results from Africa and Ecuador can only be interpreted as providing evidence that ticks *could* be the cause of these IgE antibodies because other ectoparasites or helminths could be relevant (Table II). Of note, the generally negative data from Norrbotten, Boston, and northern California argue strongly that eating food containing this oligosaccharide (eg, beef or milk) does not induce sensitization to alpha-gal.

IgE antibodies to tick proteins

Studies from both Europe and Australia have reported the presence of IgE antibodies to tick salivary proteins in sera from patients who experienced anaphylactic reactions to tick bites.[26-28] Using whole-body extracts of *A americanum* or *D variabilis*, we assayed sera with or without IgE antibodies to alpha-gal for IgE antibodies specific for tick proteins. The results show a strong correlation between IgE antibodies to *A americanum* and IgE antibodies to alpha-gal (Fig 3). The titers of IgE antibodies to tick proteins were lower than the IgE antibodies to alpha-gal. When sera were absorbed using a solid phase coated with alpha-gal, half of the sera retained more than 80% of the IgE to ticks (see Table E1 in this article's Online Repository at www.jacionline.org). Four of the 12 sera had over 80% of the IgE that bound the tick extract removed by absorption with alpha-gal–coated beads. Performing the same experiments with blank sepharose beads or as a mock experiment caused less than a 1% change in alpha-gal or *A americanum* IgE binding (data not shown). The results for *D variabilis* did not show a significant relationship with IgE antibodies to alpha-gal (see Fig E2 in this article's Online Repository at www.jacionline.org). Thus, our evidence suggests that bites from *A americanum* ticks can induce both IgE antibodies to

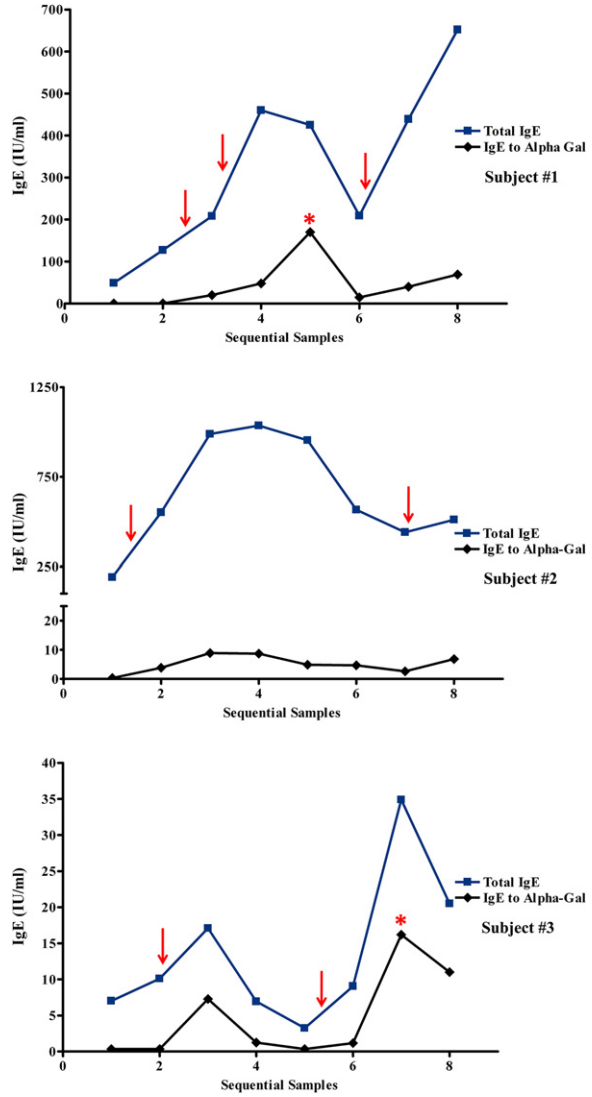

FIG 1. Time course of IgE antibodies to alpha-gal and total IgE levels collected as sequential samples after episodes of multiple tick bites *(red arrows)* in 3 subjects. The time intervals between samples vary considerably, and the specific times can be seen in Table I. Subjects 1 and 3 experienced episodes of generalized urticaria starting 3 to 4 hours after eating mutton and beef, respectively (indicated by *red asterisk*). In each case this was the first such episode.

TABLE I. Changes in IgE antibody levels* (IU/mL) after tick bites‡ in 3 subjects

Subject 1§	April 2002	October 2006	May 2007	August 2007	January 2008	February 2010	August 2010	September 2010
Dust mite	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Cockroach	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Rye grass	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Silver birch	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Trichophyton species	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Dog epithelium	**0.46**	**0.38**	**10.2**	**29.3**	**64.3**	**11.5**	**29.9**	**41.1**
Beef	**0.56**	**0.40**	**10.3**	**28.0**	**65.0**	**13.3**	**34.7**	**45.2**
Alpha-gal	**0.51**	**0.49**	**20.2**	**48.3**	**170**	**14.7**	**40.1**	**69.2**
Total IgE	49	127	208	460	425	209	439	652

Subject 2†	July 2007	June 2009	July 2009	July 2009	August 2009	January 2010	February 2010	November 2010
Dust mite	**0.62**	**0.55**	**0.68**	**1.28**	**1.26**	**0.80**	**0.65**	**0.6**
Cockroach	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Rye grass	**6.83**	**5.30**	**6.02**	**7.21**	**6.69**	**5.68**	**5.03**	**6.86**
Silver birch	**30.4**	**28.3**	**31.0**	**39.4**	**35.5**	**33.5**	**31.4**	**36.1**
Trichophyton species	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Dog epithelium	0.35	**1.21**	**2.37**	**2.46**	**2.35**	**1.28**	**1.12**	**1.63**
Beef	0.35	**1.47**	**2.53**	**3.04**	**2.59**	**1.37**	**1.25**	**2.15**
Alpha-gal	0.35	**3.88**	**8.94**	**8.71**	**4.87**	**4.67**	**2.62**	**6.84**
Total IgE	192	551	989	1036	954	566	440	510

Subject 3§	June 1996	June 1999	August 2008	August 2009	March 2010	September 2010	October 2010	November 2010
Dust mite	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Cockroach	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Rye grass	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Silver birch	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Trichophyton species	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Dog epithelium	0.35	0.35	**3.36**	**0.78**	0.35	**0.67**	**9.3**	**4.66**
Beef	0.35	0.35	**4.15**	**1.00**	0.35	**0.85**	**12.0**	**5.9**
Alpha-gal	0.35	0.35	**7.29**	**1.24**	0.35	**1.18**	**16.2**	**11**
Total IgE	7.01	10.1	17.1	6.95	3.25	9.05	34.9	20.5

*Values for serum IgE antibodies and total IgE after episodes of multiple tick bites. Values are shown for 5 allergens unrelated to alpha-gal: dust mite, cockroach, ryegrass, silver birch, and the fungus *T rubrum*. Values for IgE antibodies that are related to the oligosaccharide alpha-gal are shown in *shaded areas*.
†Subject 2 was allergic to mite, grass, and birch before tick bites.
‡The timing of episodes of multiple tick bites are shown as *vertical black lines*. In each case the bites occurred within 10 days of the subsequent blood draw.
§Subjects 1 and 3 experienced generalized hives 3 to 4 hours after eating red meat. In each case this was their first such episode and occurred when the IgE antibody titer to alpha-gal was high (subject 1: November 20, 2007; subject 3: October 10, 2010).

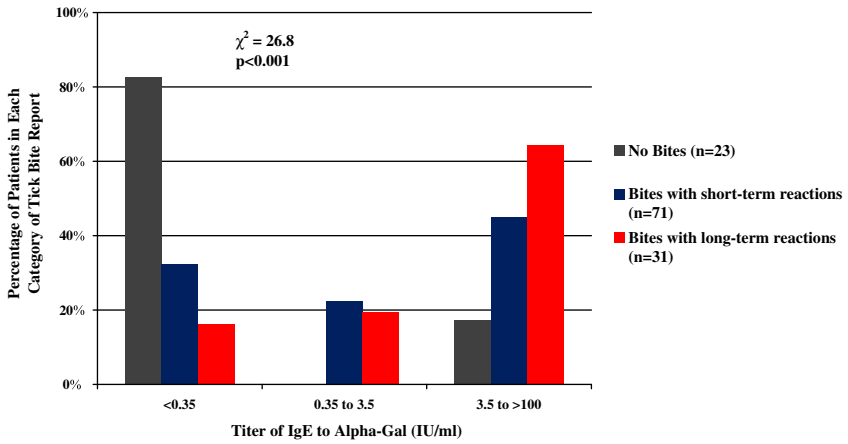

FIG 2. Prevalence of IgE antibodies to alpha-gal among 125 patients with or without histories of tick bites. Histories of bites were analyzed by length of time that reactions persisted at the site. χ^2 Analysis on 3 bite categories versus assays for IgE antibodies of greater than 0.35 IU/mL was performed.

tick proteins and IgE antibodies to alpha-gal. The sera from the inland town of Kiruna in the Norrbotten province of Sweden were uniformly negative for IgE antibodies to ticks.

DISCUSSION

The results presented here strongly suggest that tick bites are a cause, if not the only significant cause, of IgE antibody responses

TABLE II. Prevalence of IgE antibodies to alpha-gal in different populations related to local prevalence of tick bites

	Prevalence*	Percentage
Virginia clinic populations		
Patients presenting with delayed anaphylaxis to red meat†[9]	117/121 (99)	97
Asthma in the clinic‡	6/56 (3)	11
Random populations in the Southeast§		
Tennessee[5]	24/107 (11)	22
North Carolina	15/75 (9)	20
Virginia (ER)[16,17]	25/136 (8)	18
Southeastern populations, total	64/318 (28)	20
Areas where tick bites are rare		
Boston Women's Study (adults)[18]	2/341 (0)	<1
Norrbotten, Sweden (age 18 y)[19]	1/150 (0)	<1
Northern California		
Stanford University cancer patients[5]	3/49 (0)	6
Travis Air Force Base, asthmatic patients and control subjects[20]	1/117 (0)	1
University of California, San Francisco, asthmatic patients and control subjects	3/136 (1)	2
Northern California, total	7/302 (1)	2
Tropical areas where tick bites are common‖		
Kabati, Kenya (rural)[21]	100/131 (50)	76
Thika, Kenya (moderately sized industrial town)[21]	36/123 (10)	29
Esmeraldas Province, Ecuador[22]	110/295 (36)	37

*Values are the number of sera at greater than 0.35 IU/mL, and the values in parentheses are the number of sera at greater than 3.5 IU/mL. Also shown is the percentage of positive sera.
†Patients presenting to the clinic with a history of recurrent anaphylaxis, angioedema, or generalized urticaria occurring 3 to 5 hours after eating mammalian meat.[9]
‡Patients evaluated in the University of Virginia Allergic Disease clinic for asthma, including 30 with severe or moderately severe asthma.
§Three studies in which enrollment was unrelated to histories that could be related to IgE antibody responses to alpha-gal.
‖Tick bites of several species are known to be common in each of these areas, but there are also multiple helminths and other ectoparasites that could be relevant.

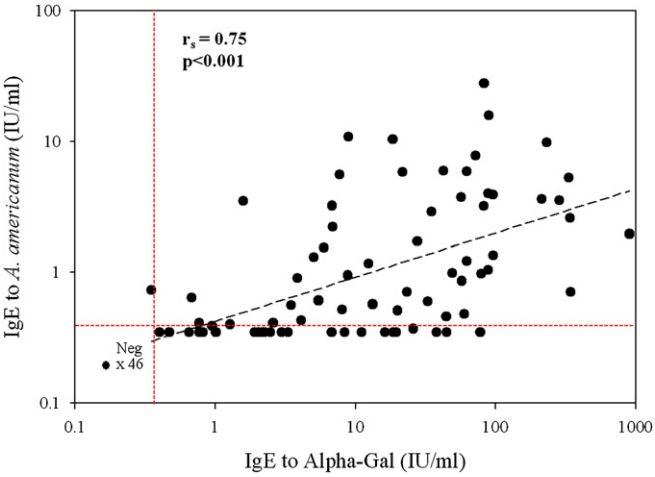

FIG 3. Correlation between IgE antibodies to alpha-gal and IgE antibodies to proteins derived from the tick *A americanum* in 125 sera obtained from patients living in Virginia.

to alpha-gal in the southeastern United States. This evidence includes following the response prospectively in 3 cases, a strong correlation with histories of tick bites, epidemiologic evidence that these antibodies are not found in regions where tick bites are rare, and correlation with IgE antibodies specific for tick proteins. As recently as January 2006, we were totally unaware of this phenomenon. Our present results in Virginia, North Carolina, and Tennessee suggest that as many as 20% of the population in these states have serum IgE antibodies to alpha-gal. In 2009-2010, delayed anaphylaxis to red meat is a common reason for presentation to the clinics of this predominantly rural or suburban area of central Virginia. Nevertheless, we do not mean to suggest that 15% of the population in these states has an allergy to mammalian meat. We are aware of many subjects with IgE to alpha-gal (and beef) who do not have reactions after eating red meat. In addition to the high prevalence, the titers of IgE antibodies to alpha-gal can be very high. In many cases, patients have greater than 100 IU/mL IgE antibodies to alpha-gal, often in conjunction with high total IgE levels. Indeed, our results suggest that tick bites can be an important cause of high total serum IgE levels. That the saliva of ticks is a potent immunogen is well known[29-31]; however, it has not previously been reported that ticks could induce IgE antibodies to a cross-reactive oligosaccharide.

Our original observation was that the distribution of anaphylactic reactions to cetuximab (Fig 4, *A*) was similar to the maximum prevalence of Rocky Mountain spotted fever (Fig 4, *B*). The major vectors of Rocky Mountain spotted fever in this region are the ticks *D variabilis* and *A americanum*, and the geographic range of *A americanum* has been expanding over the last 30 years, probably in parallel with the massive increase in the deer population (Fig 4, *C* and *D*).[13,14,32,33] Many persons are not aware that in 1950 deer had been hunted close to extinction in the southeastern United States. Indeed, at about that time, the Virginia state game commission "reintroduced" deer to sections of the Blue Ridge Mountains. Over the last 20 years, the deer population has expanded to become a major pest in suburban areas. This further increase might have been made possible by the introduction of leash laws for dogs. Our focus has been on *A americanum* because (1) 2 of our cases involved this species, (2) the cases match the known distribution of this tick (Fig 4, *C* and *D*), and (3) this species has a well-deserved reputation for being "aggressive."[14,32-34] Both adult and larval forms of this tick are very willing to bite humans, as well as other mammals. The larval ticks are about 1 mm in diameter and are generally not seen or appear as small black dots. This creates a confusion that is important in taking histories. The term used for larval ticks is "seed ticks"; however, the term "chiggers" is better known, and it is not easy to identify either seed ticks or chiggers correctly. Chiggers are correctly the larval forms of trombiculid mites, which can also bite humans but are less common than seed ticks in the region in question.[35]

Our evidence about the regional distribution of these reactions does not favor a role for *Ixodes scapularis*. Indeed, the Lyme disease literature has very few mentions of multiple bites or severe pruritic reactions to bites. This might well be because persons who consistently report itching in association with tick bites are less likely to experience an episode of Lyme disease than those who do not react against tick bites of *I scapularis*.[36] In the same study from Block Island, where Lyme disease is highly endemic, it was found that the frequency of itch increased as the number of reported tick bites increased, suggesting that tick-related itch was associated with an acquired cutaneous hypersensitivity response.[36] Of note, the larval forms of the ticks that carry

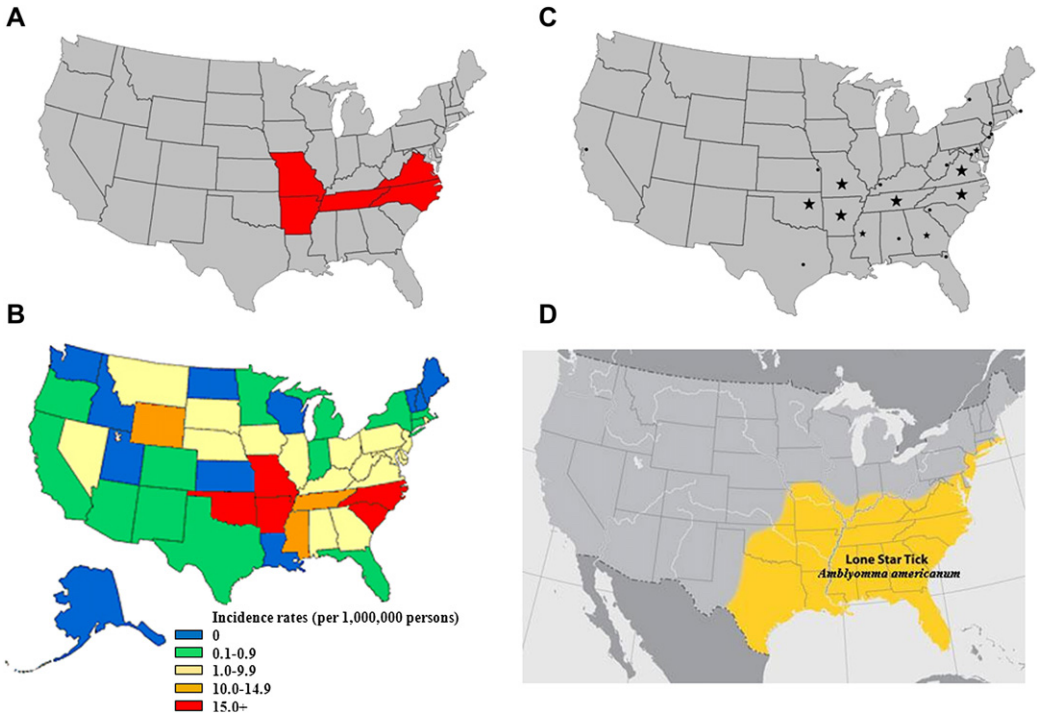

FIG 4. **A** and **B**, Comparison of the states where anaphylactic reactions to cetuximab were reported in 2006 (Fig 4, *A*) and the reported prevalence of Rocky Mountain spotted fever (Fig 4, *B*). **C,** Distribution of known cases of patients with delayed anaphylaxis to red meat whose serum contained IgE antibodies to alpha-gal. Markers indicate the number of cases: *dots* indicate single cases, *smaller black stars* indicate 5 to 24 cases within a state, and *larger black stars* indicate states with 25 or more cases. Data in Fig 4, *A*, are from Bristol-Myers Squibb, and data in Fig 4, *B*, are from the Centers for Disease Control and Prevention Web site. Numbers of cases in states with stars: Arkansas, 65; Georgia, 14; Maryland, 8; Mississippi, 5; Missouri, 30; North Carolina, 35; Oklahoma, 25; Tennessee, 40; and Virginia, greater than 200. **D,** Known distribution of the tick *A americanum* (data from the Centers for Disease Control and Prevention Web site).

Borrelia burgdorferi are not known to bite humans. In other areas of the world, different ticks have been associated with allergic reactions in humans. In Australia, bites of *Ixodes holocyclus* can precede subsequent allergic reactions to beef.[15] In Europe the pigeon tick, *Argas reflexus*, is known to cause sensitization and anaphylactic reactions to subsequent bites from ticks of the same species.[27,28] That tick has not been associated with allergic reactions to red meat, and the IgE antibodies have been shown to be specific for a protein.[28] In the Stockholm area of southern Sweden, ticks, particularly *Ixodes ricinus*, are a major problem, and IgE antibodies to alpha-gal have been reported.[37,38] At present, it seems likely that several different ticks can cause an IgE antibody response to alpha-gal; however, the behavior of the ticks (eg, propensity to bite humans) might also be important.

The severity of the pruritic symptoms that our patients report has been impressive. Although many different patterns of tick bites have been reported, 3 forms stand out. The first pattern is a few bites from adult ticks that persist for weeks or months, remaining pruritic. The most severe case of this kind was a report of 2 tick bites removed surgically 6 months after the original contact. The second pattern is repeated bites, often around the ankles, in subjects who work outside or hunt regularly. In a few cases the local reactions to the ticks have been so severe as to preclude further work outside. The third pattern is multiple bites from larval ticks, generally 10 or more, but often hundreds, which are again severely pruritic but generally do not last more than a few weeks.

We have seen high or very high (>100 IU/mL) titers of IgE antibodies to alpha-gal in patients with histories of each of these types. Surprisingly, despite the severity of pruritic reactions locally and the presence of IgE antibodies to tick proteins, we are not aware of any cases of anaphylactic reactions to subsequent tick bites among patients we have seen. There is, however, 1 case report of anaphylaxis to a tick bite in the United States.[39]

The number of cases of delayed anaphylaxis diagnosed in central Virginia and the surrounding areas has increased dramatically, suggesting that we might be looking at an epidemic. However, there were some cases of this condition seen, if not correctly diagnosed, 20 years ago; in fact, 2 patients have recently come to see us and reported that they had told us the same story 15 and 18 years previously! Equally, we know that IgE antibodies to alpha-gal were present in this area in 1988 because sera from an emergency department study at that time have recently been assayed for IgE to alpha-gal.[40]

If the incidence has increased, there are several possible causes related to ticks. There is good evidence that *A americanum* has increased its range and that its primary host has increased dramatically in numbers.[14,33] It is also possible that some factor about the tick has changed, such as a minor variation in the composition of saliva or the presence of a symbiotic organism. This species is known to carry multiple species of rickettsiae, including *Rickettsia amblyommii* and the organism responsible for human ehrlichiosis, *Ehrlichia chaffeensis*.[41,42] Although the results of absorption experiments provide clear evidence for antibodies

that can be absorbed by alpha-gal and those that cannot, we do not know the target for the IgE antibodies that bind to tick-derived proteins (Fig 3 and see Table E1). Future studies will focus on identifying the possible causes of the IgE response to ticks, which include a symbiotic organism, bystander polyclonal IgE induced in the alpha-gal response or a concomitant sensitization to tick salivary protein(s).

Taken together, the evidence pointing toward tick bites as the primary cause of IgE antibody responses to alpha-gal creates a compelling argument. In addition to the 3 cases reported in detail here, there is epidemiologic evidence that (1) these IgE antibodies are found in areas where tick bites are common, (2) the responses correlate with reports of tick bites, and (3) IgE antibodies to alpha-gal correlate with the presence of IgE antibodies to tick proteins. Furthermore, the currently known distribution of delayed anaphylactic reactions to red meat is similar to the known distribution of *A americanum* (Fig 4, *C* and *D*). Predictions are that populations of both deer and ticks will continue to increase their range, particularly in suburban areas. Thus the number of Americans exposed to these ticks and their larval forms will, in all probability, continue to increase.

We are grateful to the many physicians who have shared data with us, including Tina Hatley-Merritt, Jane Purser, Carol Wiggins, Michael Land, Marty Fiser, Dan Atkins, Phil Lieberman, Luis Matos, Saju Eapen, Dane McBride, Joey Lane, Gary Rakes, Eddie Shields, and Peter Bressler. We also thank Amy Klion at the National Institutes of Health for discussion about IgE responses to parasites and for sharing sera of patients with helminth infestation. We would also like to thank Dr Raymond Mullins for thoughtful conversations regarding the experience of meat allergy and ticks in Australia. We are grateful to Dr Robert Lane, who identified the seed ticks that bit subject 1. We are also grateful to Walter Oliveira, who has established the assay for IgE to alpha-gal in the hospital laboratory at the University of Virginia.

Key messages

- **IgE antibodies to the oligosaccharide alpha-gal are related to 2 forms of anaphylaxis that are most common in the southeastern United States (eg, anaphylaxis during cetuximab infusion and anaphylaxis after eating mammalian meat).**
- **Tick bites are directly related to the presence of these IgE antibodies as assessed prospectively, by history of tick bites, and by analyses of the areas where these IgE antibodies are found.**
- **This IgE antibody response provides a model of an ectoparasite-specific response that has significant clinical consequences.**

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APPENDIX E1. Further description of cohorts contained in Table II

Virginia, Allergy Clinic

Patients seen at the University of Virginia Allergy and Asthma Clinic with the complaints of anaphylaxis, angioedema, generalized urticaria, or asthma were enrolled. Additionally, 40 subjects seen in the clinic without these complaints (eg, immune deficiency and contact dermatitis) were enrolled as control subjects.

Tennessee

Sera (n = 107) from Vanderbilt University Medical Center included subjects with cancer who had received cetuximab, as well as subjects who were enrolled as healthy volunteers at a yearly cancer-screening event.

North Carolina

Sera from 75 hospitalized patients were chosen in May 2009 from the general medicine and surgical inpatient census at University of North Carolina Hospital. None of the patients were known to have a cancer diagnosis, and none had been exposed to cetuximab.

Virginia, Emergency Department

Sixty-six randomly selected patients with acute asthma and 70 age- and sex-matched control subjects between the ages of 18 and 50 years were recruited in the emergency department of the University of Virginia Health Sciences Center in Charlottesville. The exclusion criterion for the control subjects was acute breathlessness at the time of the study.

Boston Women's Study

Sera screened were samples from a metropolitan Boston prospective birth cohort study designed to examine relationships between exposure to indoor allergens and the development of allergic sensitization and asthma. All mothers who delivered at a large Boston hospital were approached for screening within 24 to 48 hours after delivery if they lived within Route 128 (encircling the metropolitan area), were 18 years old or older, and were able to speak English or Spanish.

Norrbotten

Sera samples were from the research program Obstructive Lung Disease in Northern Sweden. In 1996 and 2006, all children in the first-grade and second-grade classes (age 7 and 8 years) in 2 municipalities in northern Sweden were invited to participate in skin prick tests and a questionnaire study. The participation rate for skin prick tests in 1996 was 88%, and 90% of the children participated in 2006, with a similar participation rate among girls and boys and in the 2 areas. All children with any positive skin prick test responses in 4 schools in Luleå were invited to undergo a second skin prick test, and sera were taken at age 18 years for analyses of specific IgE antibodies.

Stanford University

These 49 sera samples were from subjects with cancer of the head and neck who had presented at the Stanford University Medical Center in Stanford, California.

Travis Air Force Base

These sera were obtained from 60 adult patients presenting with wheeze and 57 control subjects evaluated for a nonrespiratory complaint at Travis Air Force Hospital, Fairfield, California, within 2 weeks of the peak grass pollen counts.

University of California, San Francisco

Sera were from subjects with asthma (n = 102) or control subjects (n = 41) enrolled in studies on asthma at the University of California San Francisco.

Kenya

Sera were collected from 2 cohorts. The rural cohort was from the Mukerengu Primary School located in Kabati, an area approximately 80 km north of Nairobi. The major sources of income in Kabati are farming, raising livestock, and working for large pineapple and coffee farms. Most of the children live in thatched-roof homes. Three of 4 classes from the fourth grade were randomly selected. The more urban cohort was from the Kenyatta Primary School located in Thika, a small industrial town 50 km north of Nairobi and only 30 km from Kabati. The many factories in Thika include pineapple processing, battery manufacturing, and bread making. Most of the children from the school live in housing built by the companies in the town: 1-room dirt-floor brick houses arranged as 8 units around a courtyard (1 family per room).

Ecuador

The sera from this cohort were collected from schoolchildren aged 5 to 18 years in adjacent districts in the provinces of Pichincha and Esmeraldas. The area is subtropical/tropical rain forest at altitudes of 50 to 1000 m above sea level. Economic activities in the area are centered largely on agriculture and cattle. The study area consists of small (15-100 houses) and homogeneous (in terms of economic means, lifestyle, and living conditions) communities interconnected by dirt roads. General features of housing are wood or breeze-block walls, corrugated iron roofs, and uncovered wooden or cement floors. Cooking is with propane or wood.

Subject ID # _____

IRB #13298: Analysis of allergic reactions to the xenoantigen gal-alpha-1,3-gal in patients with spontaneous anaphylaxis

Name: _____

Address: _____

Home/Cell Phone: _____ Email Address: _____

Date of Birth: _____ Sex (circle one): M F

Race (please circle): Caucasian / African American / Hispanic / Asian / other_____

Please circle your responses to the following questions:

Have you ever had:

Hives (red, itchy welts)? Y / N

Anaphylaxis (low blood pressure, light headedness, trouble breathing)? Y / N

Have you ever required urgent care for hives (urticaria) or anaphylaxis in a:

Emergency Department (Emergency Room)? Y / N

Other site? Y / N If yes, where? _____

Do you have symptoms (itchy eyes, runny nose, trouble breathing) when:

1) Exposed to cats or dogs? Y / N 2) Mowing the lawn? Y / N 3) Seasonally? Y / N

If yes, is it in (circle all that apply): March, April, May, June, July, August, September, October

Do you have reactions when you eat certain foods? Y / N

If yes, please list which foods for each category below:

1) GI/oral (itchy mouth, tingling tongue, swelling of lips, nausea, diarrhea): _____

2) Skin (hives, flushing): _____

3) Anaphylaxis (trouble breathing, loss of blood pressure): _____

For each food listed above, please answer the following four questions (attach extra sheets if needed):

1) Please describe your symptoms:_____

2) How quickly after eating did your symptoms begin?

Please give an estimated time (in minutes or hours) _____

3) How much time elapsed between your first symptom and the maximum severity of your symptoms (please indicate in minutes or hours)? _____

4) In what year did you have your first reaction (e.g. hives or anaphylaxis)? _____

Have you had poison ivy? Y / N

If yes, (circle one) within 6 months, 2 years, 10 years?

FIG E1. Questionnaire.

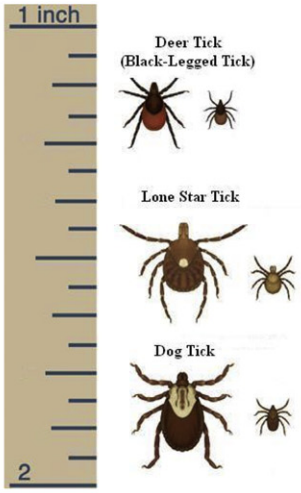
Subject ID # _____

Do you have reactions to insect bites (e.g., mosquitoes, bees, hornets, etc.)? Y / N

If yes, (circle one) within 6 months, 2 years, 10 years?

Which insect(s) and what kind of reaction? _____

Tick Bites:



Do you remember being bitten by ticks or chiggers? Y / N

If yes: Deer Ticks (top image): Y / N

If yes, (circle one) within 6 months, 2 years, 10 years?

Lone Star Ticks (middle image): Y / N

If yes, (circle one) within 6 months, 2 years, 10 years?

Hard/Wood/Dog Ticks (bottom image): Y / N

If yes, (circle one) within 6 months, 2 years, 10 years?

Seed Ticks (smaller than Deer Ticks): Y / N

If yes, (circle one) within 6 months, 2 years, 10 years?

Chiggers: Y / N

If yes, (circle one) within 6 months, 2 years, 10 years?

Do you ever have any redness, itching, or swelling around the bite? Y / N

If yes, how long does it last (circle one): days, weeks, months, years

Have you ever been diagnosed with a tick-borne illness? Y / N

If yes, circle one: Lyme disease, STARI, Rocky Mountain Spotted Fever, Erlichiosis, other _____

Medical History:

Have you wheezed in the last 5 years? Y / N

If yes, when do you usually wheeze (e.g. at night, after exercise)? _____

Have you ever been diagnosed with asthma by a doctor? Y / N

If yes: Do you use an asthma medication on a scheduled basis? Y / N

If yes, which one(s)? _____

Do you use a rescue inhaler? Y / N

If yes, how often? _____

Have you ever required oral steroids, an ER visit, or hospitalization for your asthma? Y / N

Do you have a history of allergies to medications? Y / N

If yes, please list: _____

Do you know your blood type? Y / N

If yes, circle one: A, B, AB, O

FIG E1. *(Continued)*

Subject ID # _____

Home Environment and Travel History:

Do you live in (circle one): a city, a suburb, the country?

Do you have any indoor pets (circle all that apply)? Cat, Dog, Hamster, Ferret, Gerbil, other: _____

Do you have contact with farm animals? Y / N

Are you active outdoors? Y / N

If yes, please circle all that apply: Hunting, Fishing, Hiking, Gardening, other _____

Have you lived in other areas within the US? Y / N If yes, which state(s)? _____

Have you traveled to: Mexico: Y / N, Caribbean Islands: Y / N, Other countries (please list): _____

Do you recall any GI problems when traveling? Y / N If yes, where and what type of problems?

Place: _____ Circle symptom(s): Nausea, Diarrhea, Vomiting, other _____

Place: _____ Circle symptom(s): Nausea, Diarrhea, Vomiting, other _____

Have you ever been diagnosed with a worm (helminth) infection? Y / N

Emergency Care History:

If you have received Acute or Emergency Care for urticaria (hives or "whelps"), angioedema (swelling of the lips and tongue) or anaphylaxis (hives and low blood pressure, light headedness, or trouble breathing) please answer the following:

1) How many times (circle one)? One, Two, Three, Four, more
2) Give the approximate date of each visit (month and year): _____
3) Did you ever go by ambulance to the Emergency Department for this problem? Y / N
4) Either while in the Emergency Department or while in the ambulance did you ever receive any of the following treatments?
   - Adrenaline injection (epinephrine)? Y / N / don't know
   - Antihistamine (Benadryl, etc.)? Y / N / don't know
   - Injected steroids (Solumedrol, etc.)? Y / N / don't know
   - Oral steroids (Prednisone, etc.)? Y / N / don't know
   - Intravenous fluids? Y / N / don't know
   - Hospital admission? Y / N
5) Was your blood pressure low on arrival at the Emergency Department? Y / N / don't know
6) What time of the day did you seek care (circle one)?
   Morning (6 a.m – noon), afternoon (noon - 6 p.m.), evening (6 p.m. – midnight), after midnight
7) After your care, was it clear to you what caused your urticaria, angioedema or anaphylaxis? Y / N
   If yes, please list the cause? _____
8) On any occasion, did you eat beef, pork, or lamb within 8 hours of the beginning of your reaction(s) that resulted in an Emergency Department visit? Y / N / don't know
   If yes, on how many of your ED visits do you remember eating beef, pork, or lamb prior to your Emergency Department visit (circle one)? One, Two, Three, Four, more

FIG E1. *(Continued)*

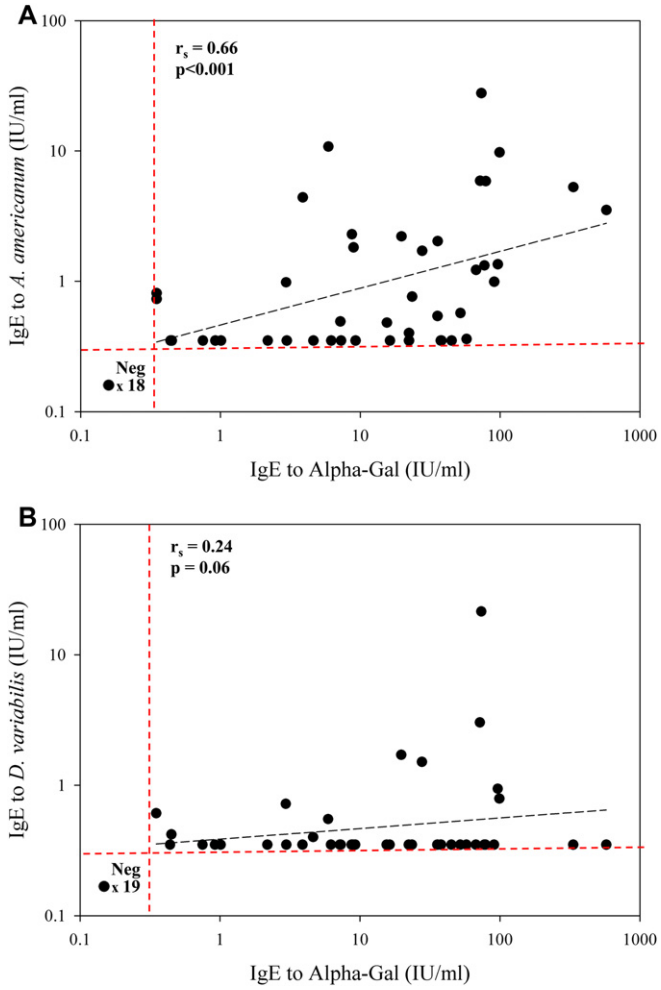


FIG E2. Results are shown for the correlation between IgE antibodies to alpha-gal and IgE antibodies to *A americanum* **(A)** and *D variabilis* **(B)**. Data are shown for the 61 sera for which assay results were available for both ticks.

TABLE E1. Absorption of alpha-gal and *A americanum*–specific IgE using solid-phase alpha-gal in subjects with IgE to alpha-gal

	Absorption with control beads*		Absorption with alpha-gal beads		
Study ID	**IgE to alpha-gal (IU/mL)**	**IgE to *A americanum* (IU/mL)**	**IgE to alpha-gal (IU/mL)†**	**IgE to *A americanum* (IU/mL)†**	**Evidence for IgE antibody to epitopes other than alpha-gal**
E036	78.4	8.40	9.87 (87%)	0.90 (89%)	−
E082	15.4	2.84	1.36 (91%)	3.20 (<5%)	+++
E177	136	14.9	12.0 (91%)	9.28 (38%)	++
E182	588	18.8	49.3 (92%)	3.64 (81%)	−
E351	61.7	5.58	3.10 (95%)	0.36 (94%)	−
E434	29.7	5.31	1.42 (95%)	4.53 (15%)	++
E454	11.3	1.95	2.23 (80%)	1.14 (42%)	+
E483	65.7	5.81	3.30 (95%)	1.12 (81%)	−
18368	6.82	3.76	1.44 (79%)	4.21 (<5%)	++
19396	7.93	9.04	1.35 (83%)	9.61 (<5%)	+++
19692	1.08	0.52	<0.35 (≥68%)	0.43 (17%)	++
19817	3.40	2.17	0.37 (89%)	2.31 (<5%)	++

*Absorption with control beads reduced alpha-gal or *A americanum* IgE binding by less than 1%.
†The number in parentheses is the percentage of specific IgE removed during absorption with solid-phase alpha-gal.