

RESEARCH LETTER

High prevalence of sIgE to Galactose- α -1,3-galactose in rural pre-Alps area: a cross-sectional study

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IgE antibodies against galactose- α -1,3-galactose (α -Gal) were recently identified in a subset of patients, who developed severe anaphylactic reactions at the first treatment with cetuximab [1]. In addition, IgE antibodies to α -Gal have also been shown to be related to delayed anaphylactic reactions after the ingestion of red meat [2]. Intriguingly, both reactions caused by cetuximab and ingestion of red meat were initially reported from the same region, a group of south-eastern states of the United States, which led to the suggestion that the cross-reactivity could originate from locally occurring biting insect or other parasites [3]. In the United States, the development of IgE antibody to α -Gal has been linked to bites from ecto-parasitic ticks, especially those of the Lone Star tick, *Amblyomma americanum* [3], whereas in Europe and Australia sensitization is associated with bites of the *Ixodes* species [4,5]. Hamsten and co-workers [5] showed that the α -Gal epitope is present in gastrointestinal tract of *Ixodes ricinus* and it involves host exposure to α -Gal during a tick bite.

The aim of this study was to investigate the prevalence of IgE antibodies to α -Gal in a general rural population in comparison with an urban population. Furthermore, we wanted to determine whether atopy, number and timing of tick bites and outdoor activities were risk factors for α -Gal sensitization. Sera for IgE anti- α -Gal measurement were obtained through a serological survey [6] undertaken in the rural village of Tramonti (426 metres above sea level, 745 inhabitants) in the Friuli Venezia Giulia region (Northeast of Italy) in 2006, to study a tick-borne encephalitis (TBE) in this setting, where inhabitants are highly exposed to bites of *Ixodes ricinus*. All members of the resident population over the age of 10 were asked to participate in the survey. A serum sample and an interview were obtained from 421 people (56.5% of the total inhabitants) (mean age = 55.2 years; m/f ratio = 1.06) and, as controls, from 80 people living in a urban area 30 kilometres away, randomly selected among the outpatients afferent

to the Pordenone Hospital matched for sex and age with the Tramonti's population (mean age = 50.1 years; m/f = 1.0). The ethics committee of the Pordenone Hospital approved the study (approval number: 98-2005), and all the individuals gave written consent for the use of their sera for the original TBE study and for further epidemiological studies. An aliquot of all the sera was stored at -80°C until tested for this study.

A questionnaire including demographic data (age, gender), a past history (yes/no) of tick bites, number of tick bites during the subject's lifetime and during the last year, and some lifestyle factors (farmer, hunter, fisher, mushrooming, mountain hiker) was administered by a physician at the time of the blood drawing. As the questionnaire was originally conceived to evaluate the risk of tick bites regarding the TBE infection, it did not include specific questions regarding allergies, previous episodes of urticaria/angioedema, anaphylaxis or documented adverse reactions to red meat or cetuximab.

All the sera were tested for sIgE to the α -Gal epitope, to beef meat (f27) and to chicken meat (f83) using the ImmunoCAP 250 automated platform (Thermo Fisher Scientific, Uppsala, Sweden) and following the manufacturer's instructions. Specific IgE towards α -Gal epitope was measured using a test containing bovine thyroglobulin bearing about 16 α -Gal moieties, which is available from Thermo Fisher Scientific for research use only. Previous studies have shown a high sensitivity and specificity for the detection of anti- α -Gal sIgE for this test, as sensitization to protein epitopes of bovine thyroglobulin is extremely rare (1,8).

For sIgE, the cut-off used for a positive reaction was $\geq 0.1 \text{ kU}_A/\text{L}$ as suggested by the manufacturer. To evaluate the atopy status of the studied population, all the sera were tested by the Phadiatop test (Thermo Fisher Scientific) and a cut-off of $\geq 0.35 \text{ kU}_A/\text{L}$ was considered a positive as suggested by the manufacturer.

The nonparametric Mann-Whitney *U*-test was used to compare continuous numerical variables between groups. The Spearman's rank test (ρ) was used to assess correlations, and the Chi-square test (with Yates' correction for continuity) was used for comparison of proportions. Medcalc 11.4.4.0 (Mariakerke, Belgium) was used for the statistical analyses, and a *P* value < 0.05 was considered significant.

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Results

Three hundred and forty-one/421 (81%) (male 98.6%; female 62.4%) of the subjects living in Tramonti and 0/80 (0%) of the subjects living in urban area reported a history of tick bites.

The prevalence of positive sIgE to α -Gal in Tramonti's population was 24.7% and proved to be significantly higher than the prevalence in the population living in an urban area (1.2%) (Table 1). Fifty percent

Table 1. Demographic data and serological characteristics of the two studied populations

Populations	Rural [†]	Urban [‡]	<i>P</i> -value*
Number of individuals (<i>n</i>)	421	80	
Males (M/F ratio)	1.1	1.0	
Median age (years)	55.2	50.1	0.473
Individuals reporting tick bites (<i>n</i>)	341	0	<0.0001
Male (<i>n</i> ; %)	213; 98.6%		<0.0001
Female (<i>n</i> ; %)	128; 62.4%		
Prevalence of IgE responses (%)			
α -Gal	24.7	1.2	<0.001
Male	33.8		<0.0001
Female	15.1		
Beef	16.9	0	<0.001
Chicken	2.4	0	0.334
Phadiatop	21.4	22.5	0.943
Lifestyle factors and prevalence of α -Gal IgE response in			
Tramonti's population	Yes	No	<i>P</i> -value
Farmer	42/163 (25.8%)	62/258 (24.0%)	0.763
Hunter	21/49 (42.9%)	83/372 (22.3%)	0.003
Fisher	10/30 (33.3%)	94/391 (24.0%)	0.358
Mushrooming	30/96 (31.2%)	74/325 (22.8%)	0.123
Mountain walker	89/347 (25.6%)	15/74 (20.3%)	0.417
α -Gal IgE response in Tramonti's population: distribution for classes of age	Number (%)	<i>P</i> -value	
<20	5/20 (25.0%)	From 0.161 to 0.958	
21–30	7/38 (18.4%)		
31–40	10/53 (18.7%)		
41–50	11/61 (18.0%)		
51–60	24/87 (27.6%)		
61–70	24/85 (28.3%)		
<70	23/77 (29.8%)		

*Comparison between the study groups was performed using the Mann–Whitney *U*-test for numerical data and the Chi-Square test for categorical data.

[†]Individuals in the rural village of Tramonti (56.5% of total inhabitants).

[‡]80 control individuals living in an urban area 30 km away from Tramonti.

of positive individuals (52/104) in Tramonti's population showed a low level of sIgE to α -Gal, between 0.1 kU_A/L and 0.35 kU_A/L, but nine subjects (2.1%) showed levels > 3.5 kU_A/L (class 3 or higher). Seventy-two/104 (69.2%) of subjects with sIgE to α -Gal $\geq$ 0.1 kU_A/L also tested positive for sIgE to beef meat. A good correlation was shown between the levels of sIgE to α -Gal and sIgE to beef meat (Spearman's ρ = 0.820; 95%CI: 0.787–0.849; *P* < 0.0001). Ten subjects were positive for sIgE to chicken meat (f83), but all of them had low levels of sIgE to α -Gal, between 0.1 kU_A/L and 0.35 kU_A/L.

The prevalence of sIgE to α -Gal positivity proved to be significantly higher in males (33.8%) than in females (15.1%, *P* < 0.0001). On the contrary, no significant differences were shown across different age groups.

Figure 1 shows the association between sIgE to α -Gal positivity and the number of reported tick bites. Subjects reporting more than five bites showed a significantly higher percentage of positivity (*P* < 0.05).

The prevalence of sIgE to α -Gal positivity proved to be significantly higher (34.9%) in subjects reporting bites during the last year than in subjects not reporting bites (13.6%) (*P* = 0.003). Also, the levels of sIgE to α -Gal were significantly higher (*P* < 0.0001) in subjects reporting bites during the last year (median value = 0.44 kU_A/L) than in subjects not reporting bites (median value = 0.27 kU_A/L).

With respect to the lifestyle factors, hunters showed a higher prevalence of sIgE to α -Gal. No significant differences were shown for other lifestyle factors.

Ninety/421 (21.4%) of subjects of the Tramonti's population and 18/80 (22.5%) of the subjects living in the urban area tested atopic on the basis of Phadiatop positivity. In Tramonti's population, the prevalence of sIgE to α -Gal positivity proved to be significantly higher in atopic (44.4%) than in non-atopic (19.3%)

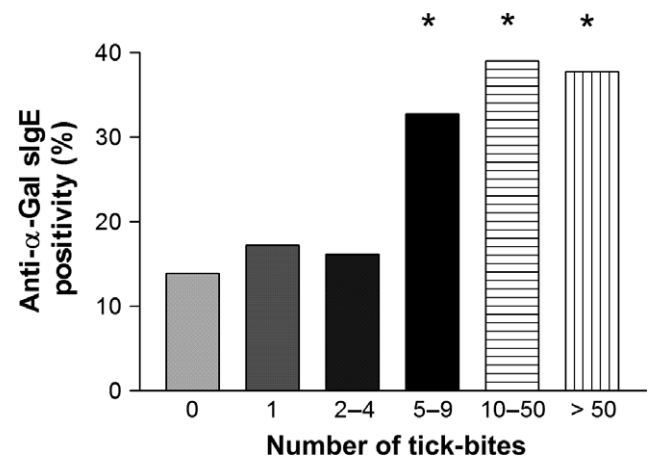


Fig. 1. Correlation between number of tick bites reported during the lifetime of the subject and prevalence of sIgE to α -Gal positivity in the Tramonti's population. **P* < 0.05.

subjects ($P < 0.0001$). No significant difference between Phadiatop positive individuals with low ($>0.1/ <0.35$ kU_A/L) or high (>0.35 kU_A/L) level of sIgE to α -Gal could be demonstrated ($P = 0.223$).

Discussion

In this study, we found that sensitization to α -Gal was significantly more common in individuals living in rural areas than in a control group living in an urban area. A quarter of the tested rural inhabitants were sensitized, half of them at a low level. Risk factors associated with increased IgE levels to α -Gal were atopy, male gender, hunting, number of tick bites and recent tick bites.

No cases of anaphylaxis to red meat and no studies describing the prevalence of sIgE to α -Gal in sera of the general population have been reported in Italy. In this study, we evaluated the prevalence of sIgE to α -Gal among inhabitants in a rural village of the pre-Alps of the Friuli Venezia Giulia region where ticks are common. Four of five reported a history of tick bites and 24.7% had sIgE to α -Gal, of which 50% were at low levels (<0.35 kU_A/L) and 2.1% at a high sIgE level above 3.5 kU_A/L. These results are in accordance with the findings described from Virginia, North Carolina and Tennessee by Commins et al. [3], but are higher than those recently reported by Gonzales-Quintela and co-workers [7]. The latter is a cross-sectional study in the general adult population in eleven municipalities in Denmark and one in Spain, and the difference might well be the high exposure to ticks in the Tramonti's population. This assumption was further supported by the finding of a low percentage of α -Gal positivity in the control subjects living in a close-by urban area.

In this study, we found a strong correlation between sensitization to α -Gal and subjects reporting tick bites. The more tick bites reported by the subject, the higher the likelihood of being IgE α -Gal positive. Also, the timing of the tick bite seems to be of importance as we observed that subjects reporting recent tick bites have a significantly higher likelihood of being sensitized to α -Gal. Hence, we can confirm that the presence of α -Gal IgE antibodies in central Europe also is associated with a history of tick bites. This statement is partially contradicted by the presence of α -Gal sensitization in subjects not reporting tick bites. A probable explanation may be that subjects are not always aware of being bitten. During the attachment and the feeding process, *Ixodes ricinus* injects a complex mixture of bioactive chemicals into the host, resulting in a painless bite. The larval ticks are about 1 mm in diameter and are generally not seen or appear as small black dots, but still may cause sensitization through their bites [3].

We found that male gender was significantly associated with sensitization to α -Gal. This confirms the

findings by Gonzales-Quintela et al. in their Danish study population, but the same authors were not able to verify this in their Spanish setting. The higher prevalence of sIgE to α -Gal in males than in females is most likely due to increased exposure to ticks in males for occupational/hobby reasons. To the best of our knowledge, outdoor activities have not previously been shown to be associated with sIgE to α -Gal. In our study, hunting was significantly correlated with α -Gal sensitization. The other outdoor activities showed a tendency of association, but did not reach statistical significance. Kubo and co-workers in Japan have previously shown that seropositivity for *Borrelia burgdorferi* induced by ticks was higher among hunters than in a control population [8].

No significant correlation regarding the prevalence of sIgE to α -Gal and age was found, confirming the data of Gonzales-Quintela et al. [7] observed in the Spanish setting of their study. Our data indirectly support the hypothesis that the α -Gal syndrome could occur in individuals of all ages.

Another important finding in our study was the higher prevalence and levels of sIgE to α -Gal in subjects bitten during the last year, confirming the observation of Kennedy et al. [9] that IgE to α -Gal appears to decrease over time. This observation is in accordance with clinical documentation of patients with a diagnosed red meat allergy that have been able to tolerate mammalian meat again after avoiding additional tick bites [9]. Our study corroborates the data of Gonzales-Quintela et al. [7] that sIgE to α -Gal positivity was consistently associated with atopy. The α -Gal epitope is present on beef proteins, and a significant correlation was found between sIgE to α -Gal and sIgE to beef meat. This finding is in accordance with the results previously reported by others [2].

One major limitation of our study is that we did not collect data regarding known symptoms related to IgE reactions to red meat. We can therefore not relate α -Gal positivity to clinical reactions. Another limitation is the age distribution, with relatively few individuals under the age of 20. The strength of this study is the substantial information regarding tick bites in general and specifically recent tick bites. We have shown a clear association between tick bites and α -Gal sensitization, but further studies evaluating the correlation between sIgE to α -Gal and allergy to red meat are needed.

In conclusion, we found a high prevalence of sIgE to α -Gal in subjects living in a rural village of the pre-Alps of Italy with high exposure to ticks. In addition, a strong relationship between the prevalence of sIgE to α -Gal and the number of tick bites reported was observed. The even higher prevalence of sIgE to α -Gal in subjects bitten in the last year further strengthens the central role of tick bites in the development and

level of IgE responses to α -Gal. Further studies are needed to evaluate the association between α -Gal sensitization and the presence of clinical symptoms due to red meat allergies. Considering the global trend with a known rise in several tick-transmitted diseases, like Lyme disease and tick-borne encephalitis, there is a substantial risk for a growing prevalence of α -Gal sensitization. This scenario also implicates increased numbers of α -Gal-related red meat allergies with an expected initial escalation in rural areas.

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Author contribution

D. Villalta and P. Martelli conceived and designed the study. L. Pantarotto, M. Conte and M. Da Re performed the laboratory measurement and carried out the statistical analyses. S. Sjolander contributed to the data analysis and to writing the manuscript. D. Villalta and MP. Borres wrote the manuscript in close collaboration with all the authors.

Conflict of interest

MP Borres and S Sjolander are employed by Thermofisher Scientific/Phadia. There are no other conflict of interests to declare by anyone of the authors.

References

- 1 Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, *et al.* Cetuximab-induced anaphylaxis and IgE specific for galactose- α -1,3-galactose. *N Engl J Med* 2008; **358**:1109–12.
- 2 Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, *et al.* Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose- α -1,3-galactose. *J Allergy Clin Immunol* 2009; **123**:426–33.
- 3 Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS, *et al.* The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose- α -1,3-galactose. *J Allergy Clin Immunol* 2011; **127**:1286–93.
- 4 Van NS, O'Connor KS, Clarke LR, Boyle RX, Fernando SL. An association between tick bite reactions and red meat allergy in humans. *Med J Aust* 2009; **190**:510–1.
- 5 Hamsten C, Starkhammar M, Tran TAT, Johansson M, Bengtsson U, Ahlen G, *et al.* Identification of galactose- α -1,3-galactose in the gastrointestinal tract of the tick *Ixodes ricinus*; possible relationship with red meat allergy. *Allergy* 2013; **68**:549–52.
- 6 Venturi G, Martelli P, Mazzolini E, Fiorentini C, Benedetti E, Todone D, *et al.* Humoral immunity in natural infection by tick-borne encephalitis virus. *J Med Virol* 2009; **81**:665–71.
- 7 Gonzales-Quintela A, Dam Laursen AS, Vidal C, Skaaby T, Gude F, Linneberg A. IgE antibodies to alpha-gal in the general adult population. Relationship with tick bites, atopy, and cat ownership. *Clin Exp Allergy* 2014; **44**:1061–8.
- 8 Kubo N, Arashima Y, Yoshida M, Kawabata M, Nishinarita S, Hayama T, *et al.* Questionnaire surveys of cases of tick bite and Lyme borreliosis in hunters in Hokkaido with reference to detection of anti-Borrelia burgdorferi antibody. *Inter Med* 1982; **31**:1163–8.
- 9 Kennedy JL, Stallings AP, Platts-Mills TA, Oliveira WM, Workman L, James HR, *et al.* Galactose- α -1,3-galactose and delayed anaphylaxis, angioedema, and urticaria in children. *Pediatrics* 2013; **131**:1545–52.