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ORIGINAL ARTICLE **Epidemiology of Allergic Disease**

IgE antibodies to alpha-gal in the general adult population: relationship with tick bites, atopy, and cat ownership

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Summary

Background The carbohydrate alpha-gal epitope is present in many animal proteins, including those of red meat and animal immunoglobulins, such as cat IgA. Systemic anaphylaxis to the alpha-gal epitope has recently been described.

Objective To investigate and compare the prevalence of alpha-gal-specific (s)IgE and its associated factors in the general adult population from two separated (Northern and Southern) European regions (Denmark and Spain, respectively).

Methods Cross-sectional study of 2297 and 444 randomly selected adults from 11 municipalities in Denmark and one in Spain. Alpha-gal sIgE was assessed by ImmunoCAP to bovine thyroglobulin. Additional assessments included a panel of skin prick test (SPT) to common aeroallergens and epidemiological factors, including the history of tick bites in the Danish series.

Results The prevalence of positive ($\geq$ 0.1 kU_A/L) sIgE to alpha-gal was 5.5% and 8.1% in the Danish and Spanish series, respectively. The prevalence of sIgE $\geq$ 0.35 kU_A/L was 1.8% and 2.2% in Denmark and Spain, respectively. Alpha-gal sIgE positivity was associated with pet ownership in both series and, particularly, cat ownership (data available in the Danish series). Alpha-gal sIgE positivity was associated with atopy (SPT positivity) in both series, although it was not associated with SPT positivity to cat or dog dander. Alpha-gal sIgE positivity was strongly associated with a history of tick bites.

Conclusions and Clinical Relevance The prevalence of alpha-gal sIgE antibodies in these general adult European populations is similarly low. The presence of alpha-gal sIgE antibodies is associated with a history of tick bites, atopy, and cat ownership.

Keywords alpha-gal, anaphylaxis, IgE, pet ownership, tick bites
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Introduction

The alpha-gal epitope (galactose-α-1,3-galactose-β-1, 4-N-acetyl glucosamine-R) is unique in mammals, being abundantly expressed on glycoconjugates of non-primates (including proteins of allergic interest in beef, pork, lamb, and cat dander), prosimians, and New World monkeys [1]. In contrast, the alpha-gal epitope is not expressed on glycoconjugates of Old World monkeys, apes, and humans. This absence of the alpha-gal epitope is the result of an evolutionary event in ancestral Old World primates, which led to the inactivation of α-1,3-galactosyltransferase, the enzyme needed for formation of alpha-gal in humans and higher mammals [1]. Instead, humans produce very large amounts of natural antibodies that specifically bind the alpha-gal epitope. These natural IgG or IgM antibodies constitute a sizeable part of circulating immunoglobulins and represent the main obstacle for xenotransplantation from species bearing the alpha-gal epitope to humans [1].

Immunoglobulin E (IgE)-mediated allergy to the alpha-gal epitope has been described, as recently reviewed [2–6]. The first indication of alpha-gal-specific IgE (sIgE)-mediated allergy came from cases of systemic anaphylaxis after the infusion of cetuximab, a chimeric mouse–human antibody that is used for cancer therapy and bears the carbohydrate epitope on the mouse Fab portion [7]. Moreover, sIgE to alpha-gal was demonstrated to underlie some cases of systemic anaphylaxis after eating red meat (including beef, pork, and lamb meat, but not chicken or fish) [8], gelatin [9], or pork

kidney [10]. Most cases have been reported in adults [2–10], but recent reports also include children [11]. Intriguingly, anaphylaxis symptoms may be substantially (3 to 6 hours) delayed post-ingestion [2–10]. The alpha-gal epitope, although present in some aeroallergens of animal origin including IgA in cat dander [12], is not associated with asthma [13, 14]. Initial cases of anaphylaxis to alpha-gal were described in the USA, and epidemiological evidence indicated a close relationship with previous exposure to tick bites [7, 15]. In Australia, a relationship between tick bite exposure and meat allergy was in fact previously suggested [16]. In Europe, cases of delayed anaphylaxis after red meat intake in relation to IgE-mediated alpha-gal sensitization were subsequently described [17–23]. Species of *Ixodes* seem to be responsible ticks in Europe and Australia, whereas *Amblyomma americanum* seems to be vector in the USA [3, 5, 15, 16, 18]. Furthermore, the alpha-gal epitope has been identified in the gastrointestinal tract of *Ixodes ricinus* [21]. Alpha-gal sensitization is less common among individuals harbouring the blood group B, which is structurally similar to the alpha-gal epitope [22, 24].

The prevalence of specific allergic sensitization may vary depending on the geographical region considered. The knowledge of the prevalence of sIgE to a given allergen in general populations of asymptomatic individuals is of foremost importance to properly interpret positive values in a clinical setting. In addition, studies in general populations may offer an insight into the mechanisms of allergic sensitization. The aim of the present study was to investigate the prevalence of and associated factors to the presence of alpha-gal sIgE in general adult populations from two separated (Northern and Southern) European regions (Denmark and Spain, respectively).

Methods

Study design

The present study included two cross-sectional surveys, from Copenhagen, Denmark, and A-Estrada, Spain. Both studies included serum alpha-gal sIgE determination, skin prick tests to a panel of relevant allergens, and structured questionnaires as follows:

The Danish study was based on the 5-year follow-up of the Health 2006 cohort. A detailed description of the baseline examination has been published elsewhere [25]. The participants in the baseline Health 2006 cohort were drawn as a random sample from the background population aged 18–69 years, living in 11 municipalities in the south-western part of suburban Copenhagen. A total of 3471 individuals (44.7%) entered the study and participated in the health examinations, which took place between June 2006 and June 2008. In 2011–12, participants in the baseline Health 2006 were invited for a 5-year follow-up examination including essentially the same study protocol. A total of 3405 were eligible for invitation (21 had emigrated and 45 died). A total of 2308 (45.8% men) agreed to participate and were re-examined between November 2011 and November 2012. The median age was 55.7 (range 24–76) years. Eleven participants did not have valid sIgE to alpha-gal measurements, thus 2297 individuals form the basis of this study.

The Spanish study was based on the A-Estrada survey. A detailed description of the study design has been published elsewhere [26]. The participants were drawn as an age-stratified random sample from the background population aged > 18 years, living in a single municipality (n = 19 346) in the north-western part of Spain. About a quarter of the population lives in the main A-Estrada village, and the remainder lives in a rural environment. From the randomly selected sample (n = 720), 65% of individuals consented to participate in the study. Thus, a total of 469 (43.9% men) were examined between January 2000 and December 2001. The median age was 54.0 (range 18–92) years. Twenty-five participants did not have valid sIgE to alpha-gal measurements, thus 444 individuals form the basis of this study.

Structured questionnaires

The structured questionnaire was self-administered in the Danish study and was administered by a physician in the Spanish study. Both studies included basic demographic data (age, gender) and lifestyle factors (smoking and alcohol consumption). Alcohol consumption was evaluated as the number of standard drinking units regularly consumed per week [25, 26]. Both studies investigated the presence of pets at home (yes/no), although only the Danish study differentiated between cat and dog. The presence of livestock (cows, sheep, pigs) near the home (yes/no) was investigated in the Spanish study, which included a rural area. The Danish study (but not the Spanish study) included a specific question regarding a past history of tick bites (yes/no).

No study asked specifically for delayed anaphylaxis to red meat. The Danish study included the question 'Have you ever experienced itching in your mouth, throat, on your tongue, or lips when ingesting certain foods?' (yes/no), and 'If yes, which foods?' The response options were apple, peach, kiwi fruit, pineapple, strawberry, celery, hazelnut, peanuts, orange, tomato, and other. The Spanish study included a general question about allergy 'Do you have any allergy?' (yes/no). If yes, individuals were asked about specific allergies.

Skin prick tests

Both studies included SPT to a panel of relevant allergens in the respective area. The Danish study included mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), pollens (birch, timothy grass, and mugwort), moulds (*Cladosporium herbarum* and *Alternaria alternata*), and animal dander (cat, dog, and horse) (Soluprick SQ system, ALK-Abelló, Hoersholm, Denmark). The Spanish study also included mites (*Dermatophagoides pteronyssinus, Lepydoglyphus destructor,* and *Tyrophagus putrescentiae*), pollens (birch, rye grass, plantain, and pellitory), moulds (*Cladosporium herbarum* and *Alternaria alternata*), and animal dander (cat and dog) (ALK-Abelló, Madrid, Spain).

A negative control and a positive control (10 mg/mL histamine) were included in both studies. If the wheal at the positive control site was less than 3 mm, the test result was considered invalid. A positive SPT was defined as a wheal of 3 mm or more after subtraction of the negative control site. The Danish study considered the mean wheal diameter, and the Spanish study only considered the largest diameter. Individuals with at least one positive SPT were considered atopic.

Total serum IgE

Determinations of total IgE (Immulite™ chemiluminescent enzyme immunoassay, Diagnostic Products Corporation [at present, Siemens Diagnostics], Los Angeles, CA, USA) were only available in serum samples from the Spanish study. Total serum IgE levels in this population have been previously reported [26].

Serum specific IgE to the alpha-gal epitope

In the Danish study, the serum samples were analysed for sIgE without prior freezing. In the Spanish study, sera were stored frozen until tested. All sera were tested for sIgE to bovine thyroglobulin using the UniCAP 250 system (Phadia Diagnostics, Uppsala, Sweden [at present, Thermo Fisher Scientific]). The CAP sIgE test to bovine thyroglobulin is sold by the manufacturer as a marker of alpha-gal sensitization for research purposes. Previous studies demonstrated that preincubation with bovine thyroglobulin inhibits alpha-gal sIgE reactivity [14]. Given that sensitization to protein epitopes in bovine thyroglobulin is extremely rare and that bovine thyroglobulin is heavily 'decorated' with alpha-gal molecules [14, 27], the detection of sIgE to bovine thyroglobulin can be used as a marker of alpha-gal sensitization. To confirm this, we compared the titres of alpha-gal sIgE with those of bovine thyroglobulin sIgE in a selected group of sera with a wide array of alpha-gal sIgE. Sera for this preliminary analysis were not part of the present series but were selected from previous studies and included some patients with delayed anaphylaxis to meat [18]. For this analysis, the streptavidin CAP technique was used as previously described [7, 14, 15, 18]. The streptavidin CAP (o212, Thermo Fisher Scientific) is commercially sold as a tool for coupling of biotinylated allergens and detection of IgE antibodies to new allergens. Briefly, 50 μg of biotinylated alpha-gal (galactose-α-1,3-galactose-β-1,4-N-acetyl glucosamine-β-spacer biotin [product code 02-079], Glycotech Corporation, Gaithersburg, Md, USA) was added to each CAP for 60 min at 37°C before adding undiluted serum, and results were measured in a ImmunoCAP-100 analyser (Phadia). The results of alpha-gal sIgE using the streptavidin CAP technique were strongly correlated with those obtained with the ImmunoCAP test to bovine thyroglobulin (Table S1). To further prove the specificity of the assay, we investigated the potential inhibition of bovine thyroglobulin sIgE reactivity after preincubation with the alpha-gal epitope in two sera from patients delayed anaphylaxis to meat and high-titre sIgE, as previously described [18]. Briefly, 150 μL of serum was preincubated overnight with 150 μL of biotinylated alpha-gal (as above) diluted in phosphate buffered saline at four different dilutions (0, 10, 100, and 300 μg/mL). Inhibition experiments showed that alpha-gal almost completely inhibited bovine thyroglobulin sIgE reactivity (Figure S1). Thus, bovine thyroglobulin sIgE was used for estimation of alpha-gal sIgE. The measuring range with this method is 0.1–100 kU_A/L, and the manufacturer considers values ≥ 0.1 kU_A/L as positive. Individuals with sIgE to bovine thyroglobulin ≥ 0.1 kU_A/L were therefore considered as having alpha-gal reactivity for epidemiological purposes.

Ethical issues

All individuals consented to participate in the studies, which were approved by the corresponding (Danish and Spanish) Institutional Review Boards (Ethics Committee of the Capital Region of Denmark, code H-3-2011-081, and Regional Ethics Committee, Galicia, Spain, code 2007/023, respectively) and conformed to the current Helsinki Declaration.

Statistical analyses

The chi-square and the Fisher's exact tests were, where appropriate, used to investigate the association between categorical variables. The Kruskal–Wallis test was used to compare continuous numerical variables between groups. The Spearman's rank test was used to assess correlation. Logistic regression was used for multivariate analysis of factors associated with positivity to alpha-gal sIgE antibodies. All variables were forced to enter the equation in regression models.

Results

The prevalence of alpha-gal sIgE in the Danish and Spanish series is presented in Fig. 1. The prevalence of positive (≥ 0.1 kU_A/L) sIgE to alpha-gal was 5.6% (95% CI 4.6–6.5%) and 8.1% (95% CI 5.6–10.6%) in the Danish and Spanish series, respectively. The prevalence of alpha-gal sIgE ≥ 0.35 kU_A/L (the classical threshold for positivity) was 1.8% (95% CI 1.3–2.4%) and 2.2% (95% CI 0.87–3.5%) in the Danish and Spanish series, respectively. Individual data of cases with alpha-gal sIgE ≥ 0.35 kU_A/L are represented in the Table S2. Titres were generally low. Only 8 individuals in the Danish series (0.35%, 95% CI 0.11–0.59%) and none in the Spanish series showed alpha-gal sIgE levels > 3.5 kU_A/L (class 3 or higher).

Table 1 displays a comparison of demographic, epidemiological, and immunological characteristics among individuals across different levels of alpha-gal sIgE. Positivity of alpha-gal sIgE was associated with older age and male sex in the Danish series, but these associations were not confirmed in the Spanish series. Alcohol consumption was associated with alpha-gal sIgE positivity in the Spanish series, but this was not confirmed in the Danish series. In the Spanish series, which included individuals from urban and rural areas, alpha-gal sIgE positivity tended to be associated with living in a rural habitat and having livestock facilities in the neighbourhood (*P* for trend, 0.02 in both cases; Table 1). These associations could not be investigated in the Danish series due to the urban study setting.

Alpha-gal sIgE positivity, particularly sIgE concentrations ≥ 0.35 kU_A/L, was consistently associated with pet ownership in both the Danish and the Spanish series (Table 1). The Danish series, which allowed for differentiation among types of pets, showed that having a cat at home was specifically associated with alpha-gal sIgE positivity. Indeed, having pets different from cat was not associated with alpha-gal sIgE positivity (data not shown). In the Danish study, a history of tick bites was strongly associated with alpha-gal sIgE positivity (Table 1). In multivariate analyses, both history of tick bites and pet ownership (specifically, cat ownership in the Danish series) were independently associated with increased risk of alpha-gal sIgE positivity (particularly when using the ≥ 0.35 kU_A/L cut-off) after adjusting for age, gender, smoking, alcohol consumption, and atopy status (Table 2). A history of tick bites was the strongest predictor for alpha-gal sigE positivity. Among the 8 individuals with alpha-gal sIgE > 3.5 kU_A/L, only 3 had pets at home, whereas 7 reported a history of tick bites (Table S2).

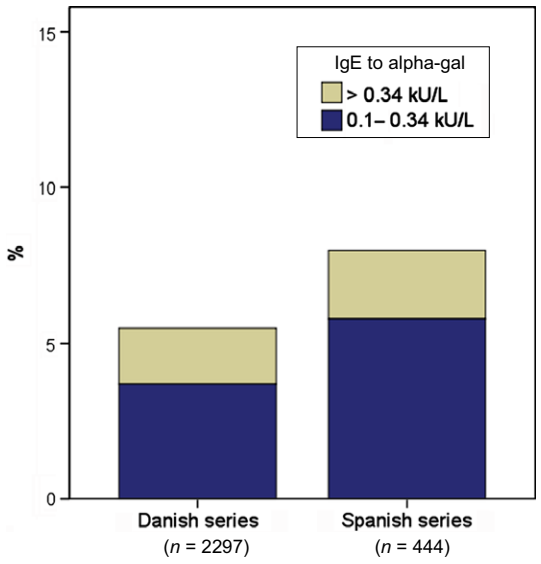

Fig. 1. Prevalence of positive alpha-gal-specific IgE in the studied populations.

Alpha-gal sIgE positivity was consistently associated with atopy (SPT positivity), in both the Danish and Spanish series (Table 1). This association was independent of potential confounders (Table 2). Similarly, alpha-gal sIgE positivity was associated with high total serum IgE concentrations (only available in the Spanish study) (Table 1). The distribution of positive specific SPT between individuals with and without alpha-gal sIgE positivity (Table 3) showed that positive SPT to mites was consistently associated with alpha-gal sIgE positivity. The association between pollen SPT reactivity and alpha-gal sIgE was less consistent. Alpha-gal sIgE was not significantly associated with SPT positivity to either dog or cat dander, in either series, although it was associated with SPT positivity to horse dander, which was only tested in the Danish series (Table 3).

In the Danish study, no participant reported red meat as the potential cause of oral allergy symptoms as assessed in the questionnaire. In the Spanish study, two individuals reported systemic reactions to hymenoptera venom, and a further two individuals reported systemic reactions to penicillin. All these individuals showed undetectable levels of alpha-gal sIgE. No participant reported red meat as the potential cause of allergy as assessed in the questionnaire.

Discussion

The present study shows that a small proportion of adult individuals from the general population in Northern Europe (Copenhagen, Denmark) and Southern Europe (A-Estrada, Spain) have sIgE to the carbohydrate epitope alpha-gal. The overall prevalence of detectable (≥ 0.1 kU_A/L) alpha-gal sIgE was slightly higher in Spain than in Denmark (8.1% vs. 5.6%, respectively). However, the prevalence of alpha-gal sIgE ≥ 0.35 kU_A/L, the classical threshold for positivity, was similar in

Table 1. Demographic data, lifestyle factors, and immunological characteristics of individuals across different levels of IgE to alpha-gal (bovine thyroglobulin)

	Danish series				Spanish series			
Factor	<0.1 kU_A/L	0.1–0.34 kU_A/L	≥ 0.35 kU_A/L	*P*-value	< 0.1 kU_A/L	0.1–0.34 kU_A/L	≥ 0.35 kU_A/L	*P*-value
Sex (male)	978/2169 (45)	54/86 (63)	23/42 (55)	0.002	179/408 (44)	13/26 (50)	5/10 (50)	0.778
Age (years)	56 (46–65)	58 (48–67)	62 (48–67)	0.035	55 (38–70)	51 (31–66)	56 (45–72)	0.753
Alcohol consumption (units/w)	4 (0–10)	3 (0–9)	4 (0–7)	0.792	2 (0–14)	7 (0–16)	6 (0–49)	0.088
Current smoking (daily)	276/2117 (13)	11/83 (13)	2/42 (5)	0.283	83/408 (20)	9/26 (35)	3/10 (30)	0.182
Habitat (rural)	NA	NA	NA	NA	301/408 (74)	22/26 (85)	10/10 (100)	0.084
Pets (any kind of pet at home)	734/2148 (34)	17/84 (20)	19/41 (46)	0.007	297/408 (73)	24/26 (92)	10/10 (100)	0.015
Pets (cat in the home)	275/2148 (13)	4/84 (5)	13/41 (32)	< 0.001	ND	ND	ND	NA
Livestock (near the home)	NA	NA	NA	NA	294/408 (72)	21/26 (81)	10/10 (100)	0.096
Known previous tick bite	612/2100 (30)	30/85 (37)	31/41 (76)	< 0.001	ND	ND	ND	NA
Atopy (SPT positivity)	672/2146 (31)	33/84 (39)	19/42 (45)	0.052	98/408 (24)	15/26 (58)	3/10 (30)	< 0.001
Total serum IgE (IU/mL)	ND	ND	ND	NA	51 (20–114)	338 (153–877)	351 (101–486)	< 0.001

ND, no data; NA, not applicable.

Data are absolute numbers (*n*/*N*) and percentages (within parentheses) or medians and interquartile ranges (within parentheses). Percentages were approximated to the nearest entire value. *P*-values were obtained with the chi-square and Kruskal–Wallis tests as appropriate.

Table 2. Multivariate analysis (logistic regression) of factors associated with IgE to alpha-gal (bovine thyroglobulin)

	Alpha-gal sIgE ≥ 0.1 kU_A/L		Alpha-gal sIgE ≥ 0.35 kU_A/L	
Factor	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Danish series				
Age (years)	1.027 (1.010–1.044)	0.001	1.050 (1.018–1.084)	0.002
Sex (male)	1.919 (1.294–2.848)	0.001	1.478 (0.751–2.911)	0.259
Current smoking (yes)	0.823 (0.461–1.470)	0.510	0.344 (0.081–1.453)	0.147
Alcohol consumption (units/w)	0.977 (0.953–1.001)	0.063	0.987 (0.946–1.030)	0.540
Pets (cat in the home)	1.164 (0.668–2.028)	0.592	3.774 (1.810–7.870)	< 0.001
Atopy (SPT positivity)	1.569 (1.063–2.315)	0.023	2.047 (1.046–4.003)	0.036
Known previous tick bite (yes)	2.760 (1.896–4.017)	< 0.001	8.467 (3.973–18.04)	< 0.001
Spanish series				
Age (years)	1.014 (0.992–1.037)	0.215	1.018 (0.977–1.060)	0.397
Sex (male)	0.703 (0.304–1.624)	0.409	0.571 (0.119–2.741)	0.484
Current smoking (yes)	2.492 (0.986–6.302)	0.054	1.863 (0.350–9.921)	0.466
Alcohol consumption (units/w)	1.022 (1.006–1.038)	0.006	1.025 (1.005–1.046)	0.015
Pets (dog or cat at home)	7.617 (1.737–33.40)	0.007	NA	NA
Atopy (SPT positivity)	3.720 (1.727–8.014)	< 0.001	1.301 (0.308–5.500)	0.720

Models were adjusted for all listed variables. Smoking, pet owning, and atopy were introduced as binary variables (1 = yes, 0 = no [reference category]). Age and alcohol consumption were introduced as continuous numeric variables. Complete data were available for 2094 individuals in the Danish series and 444 individuals in the Spanish series. OR, odds ratio; CI, confidence interval; sIgE, specific IgE; SPT, skin prick test; NA, not applicable because all individuals with alpha-gal sIgE ≥ 0.35 kU_A/L in this series (n = 10) had pets at home.

Spain (2.2%) and in Denmark (1.8%). This similarity is remarkable given the differences in the two populations, not only geographical but also regarding age and habitat, with participants being older and more frequently living in a rural setting in the Spanish than in the Danish sample. To the best of our knowledge, no similar previous study has been conducted in general populations. In selected samples of control individuals, the prevalence of alpha-gal sIgE ≥ 0.35 kU_A/L ranged from 0.6% [7] to 19% [14] in the USA, depending on geographical differences and probably on different levels of exposure to tick bites. The present study confirms that a history of tick bites is strongly associated with alpha-gal sIgE positivity. About a third of individuals in the Danish population reported previous tick bites. Among individuals with positive (≥ 0.35 kU_A/L) alpha-gal sIgE, nearly three quarters reported a history of tick bites. Furthermore, a history of tick bites was the strongest predictor of alpha-gal sIgE positivity in multivariate analyses. The questionnaire in the Spanish study

Table 3. Skin prick test positivity of individuals with and without positive ($\geq$ 0.1 kU_A/L) IgE to alpha-gal (bovine thyroglobulin)

	Danish series (n = 2297)			Spanish series (n = 444)		
	Alpha-gal negative (n = 2146)	Alpha-gal positive (n = 126)	P-value	Alpha-gal negative (n = 408)	Alpha-gal positive (n = 36)	P-value
Mites						
Dermatophagoides pteronyssinus	213 (10)	22 (17)	0.006	53 (13)	10 (28)	0.015
Dermatophagoides farinae	182 (8)	18 (14)	0.025	ND	ND	NA
Lepydoglyphus destructor	ND	ND	NA	50 (12)	10 (28)	0.009
Tyrophagus putrescentiae	ND	ND	NA	80 (20)	14 (39)	0.007
Pollens						
Birch	348 (16)	31 (25)	0.014	13 (3)	3 (8)	0.132
Grass (timothy)	371 (17)	27 (21)	0.234	ND	ND	NA
Grass (rye)	ND	ND	NA	24 (6)	3 (8)	0.555
Mugwort	165 (8)	14 (11)	0.165	ND	ND	NA
Plantain	ND	ND	NA	14 (3)	5 (14)	0.013
Pellitory	ND	ND	NA	6 (1)	2 (6)	0.131
Moulds						
Cladosporium spp.	13 (1)	2 (2)	0.200	0 (0)	1 (3)	0.081
Alternaria spp.	50 (2)	6 (5)	0.125	5 (1)	1 (3)	0.400
Animal dander						
Horse dander	32 (1)	7 (6)	0.004	ND	ND	NA
Dog dander	232 (11)	20 (16)	0.078	4 (1)	0 (0)	0.999
Cat dander	223 (10)	15 (12)	0.589	1 (0)	1 (3)	0.156

ND, no data; NA, not applicable.

Data are absolute numbers and percentages (within parentheses). Percentages were approximated to the nearest entire value. Tests for difference are chi-square or Fisher's exact test.

did not contain items related to tick bites because it was initiated in 2000, prior to the first reports of their potential involvement in alpha-gal IgE sensitization [7, 15, 16, 21]. In an ongoing study in the same municipality, the history of previous tick bite is recorded in less than 10% of the population (unpublished observation).

Our studies show that alpha-gal sIgE positivity is consistently and independently associated with atopy (SPT positivity) and pet ownership, and the Danish study further showed that the latter was specifically related to having a cat at home, and more evident for sIgE concentrations $\geq$ 0.35 kU_A/L. This association was not previously reported and should be interpreted with caution. Among patients with idiopathic anaphylaxis from Virginia (USA), a region with a high exposure to tick bites, alpha-gal sIgE was similarly high and frequent among patients with and without a cat at home [14]. The alpha-gal epitope is present in cat IgA (Fel d 5) and cat dander [12, 28]. The alpha-gal epitope is also present in IgM from cat and additional mammals [29]. Atopy renders individuals prone to aeroallergen sensitization, and therefore, it could be argued that airborne exposure to cat dander could contribute to alpha-gal sensitization. Pets in the home may increase the risk of allergic sensitization [30], although the role of cat ownership on cat allergy is a matter of debate [31]. Contrary to Fel d 1, the major cat allergen, alpha-gal is not airborne in homes with a cat [14]. It should be noted that alpha-gal sIgE positivity was not associated with positive SPT to cat dander in the present studies. Similarly, alpha-gal sIgE positivity was very rare among young individuals with cat sensitization from Northern Sweden [14]. Thus, airborne exposure has not been proven as a route for alpha-gal sensitization. Furthermore, alpha-gal sensitization is not associated with asthma [14]. Hypothetically, cat scratches could be an additional route for alpha-gal sensitization. Alternatively, the association between cat ownership and alpha-gal sIgE positivity could be biased by confounding, that is, cat exposure could be a marker of a yet unidentified causal determinant. Some pets could harbour ticks, but alpha-gal sensitization in pet owners was independent of tick bites. Pets can also harbour helminth parasites, which have been suggested as a source of alpha-gal sensitization [13, 14]. *Toxocara* spp. (*T. canis* and *T. catis* from dog and cat, respectively) is a geohelminth with high seroprevalence in some populations, including the Spanish population [32]. However, there was no association between *Toxocara* exposure (as demonstrated by serum antibodies) and alpha-gal sIgE positivity in the Spanish series (data not shown). Further studies are needed to confirm the association between alpha-gal IgE sensitization and cat ownership and to elucidate its potential mechanisms.

Strengths of the studies include the population-based random selection of adult individuals of both sexes and

a broad age range. Admittedly, the studies have limitations, in addition to those inherent to the cross-sectional design for inference of causality. The Spanish study did not include questions about tick bites and did not differentiate between cats and dogs regarding pet ownership, but the overall findings are consistent with those of the Danish study and add external validity to it. Regarding outcomes, we did not measure sIgE to the isolated alpha-gal epitope, but to a glycoprotein (bovine thyroglobulin) that bears alpha-gal epitopes and rarely induces IgE sensitization [14, 27]. This strategy is widely accepted and used, in an analogous fashion, to investigate IgE reactivity to plant/invertebrate carbohydrates (cross-reacting carbohydrate determinants) using glycoproteins that rarely induce sensitization, such as bromelain, horseradish peroxidase, or ascorbate oxidase [33]. Furthermore, in preliminary experiments, we observed a good correlation between alpha-gal sIgE and bovine thyroglobulin sIgE, and we confirmed that bovine thyroglobulin sIgE reactivity is inhibited by preincubation with alpha-gal, thus confirming the validity of the method. Both the Danish and Spanish questionnaire included items regarding existing allergies, but there were neither specific questions regarding meat allergy nor delayed urticaria or anaphylaxis. In the Spanish study, individuals with alpha-gal sIgE $\geq$ 0.35 kU_A/L were retrospectively (in 2014) asked about urticaria, anaphylaxis, and their potential relationship to certain foods adopting a previously reported specific questionnaire [15]. There was no history of systemic anaphylaxis in their clinical records. A total of 7 of the 10 positive individuals could be contacted, and none of them referred systemic reactions to meat (data not shown). However, alpha-gal-induced red meat allergy is typically delayed after ingestion, and the usual allergy history-taking can be misleading. Thus, we cannot exclude that some positive individuals are actually symptomatic cases in the present studies, which cannot serve to determine the prevalence of alpha-gal sIgE-mediated meat allergy.

From a clinical standpoint, the prevalence of a given disease and the prevalence of positive results of a diagnostic test in the general population are key features to interpret the predictive values of the test. The prevalence of alpha-gal-mediated red meat allergy in the studied areas is unknown, and it is probably low, although some cases have been described [18]. According to our results, the prevalence of alpha-gal sIgE antibodies in these southern and northern general adult European populations is low. Furthermore, with some exceptions, the concentrations of alpha-gal sIgE antibodies in these asymptomatic individuals are also low. Thus, high levels of alpha-gal sIgE in patients with clinical suspicion of red meat allergy would have a high positive predictive value. From a mechanistic standpoint, the present study shows that IgE-mediated alpha-gal sensitization is not only related to tick bites, but also to atopy and cat ownership. Further studies are needed to ascertain the mechanisms underlying these findings.

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Conflicts of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Correlation between IgE to biotinylated alpha-gal and IgE to bovine thyroglobulin in selected serum samples.

Table S2. Individual data of participants with IgE to alpha-gal (bovine thyroglobulin) ≥ 0.35 kU_A/L.

Figure S1. Inhibition of IgE reactivity to bovine thyroglobulin after overnight preincubation with alpha-gal in sera from two patients (Pt) with systemic anaphylaxis to red meat.