

ORIGINAL ARTICLE

Foodborne and orofecal pathogens and allergic sensitization: EuroPrevall-International Cooperation study

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Keywords

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Abstract

Background: An inverse association between markers of exposure to foodborne and orofecal pathogens and allergic sensitization has been reported. However, the findings of epidemiological studies have not been consistent. This study investigated the relationship between antibodies to hepatitis A, *Toxoplasma gondii* and salmonella and allergic sensitization to food and aeroallergens in children from different geographical areas.

Methods: Specific IgE and/or skin prick testing against food and aeroallergens were measured in children from 6 to 12 years of age residing in Greece, the Netherlands, China, India and Russia. Seropositivity to the three pathogens was measured, and data on potential confounders were collected using questionnaire.

Results: Data from 800 children (126 from Athens; 248 from Utrecht; 110 from Hong Kong; 119 from urban Tomsk; and 197 from rural Tomsk) could be analysed. The highest percentage of positive serology to salmonella was found in Hong Kong (46.4%), to *T. gondii* in urban Tomsk (13.4%) and to hepatitis A in Athens (71.2%). Although not significant, *T. gondii* seropositivity tends to be negatively associated, and hepatitis A seropositivity tends to be positively associated with allergic sensitization.

Conclusion: Inconsistent associations were observed between allergic sensitization to food and aeroallergens and markers of exposure to two common foodborne pathogens. The association with *T. gondii* tends to be negative, consistent with the 'hygiene hypothesis', but the association with hepatitis A tends to be positive. Taken together, there is no clear evidence that past exposure to foodborne and orofecal pathogens protects against allergic sensitization to food or aeroallergens.

Epidemiological data have shown that the prevalence of allergic diseases has markedly increased in recent decades (1). The hygiene hypothesis, originally put forward to explain lower levels of hay fever in children with many siblings (2), has been suggested as an explanation for the increasing prevalence of allergy. It has been hypothesized that increasing living

standards and hygienic conditions have resulted in decreasing exposure to infections in early life, thereby leading to an altered immunological development. This might be reflected in an imbalance of the immune system, in terms of its reactivity and cytokine profiles, such that it favours the development of allergic disorders (3–5). Epidemiologic studies investigating the

association between foodborne and orofecal pathogens and allergic disorders have shown inconsistent results. While some studies on (young) adults have supported the hygiene hypothesis and found protective effects of hepatitis A (6,7), *Toxoplasma gondii* (6–8) and *Helicobacter pylori* (6) on allergic sensitization and allergic diseases, others failed to find such association for *T. gondii* or hepatitis A (9). Studies on school-aged children found negative associations for *T. gondii* (10) and *H. pylori* (11), but not for hepatitis A (10, 12). Pelosi et al. (13) examining hospital records and children hospitalized found a protective effect of salmonella on respiratory allergies, but past exposure to salmonella in relation to allergies has not been explored. Furthermore, the effect of exposure to these foodborne and orofecal pathogens on sensitization to food allergens has not been investigated at all.

This study is part of the EuroPrevall project (www.europrevall.org), a multicentre international study evaluating the prevalence of food allergies. In each participating country, the same standardized methodology and laboratory measurements were used. European and non-European countries were involved to represent areas with different lifestyles and variable exposure to micro-organisms and parasites. The participating countries represent a diverse sample of the world's population, which provides an opportunity to test whether exposure to certain pathogens protects against the development of allergic sensitization. The aim of this study was to investigate the effect of markers of previous exposure to hepatitis A, *T. gondii* and salmonella as judged by specific serum IgG on allergic sensitization to food and aeroallergens in school-aged children living in Utrecht (the Netherlands), Athens (Greece), Hong Kong (China), Bangalore and Mysore (India), and urban and rural Tomsk (Russia).

Methods

Study population and design

The EuroPrevall study is a multidisciplinary research project looking at 'The prevalence, cost and basis of food allergy in Europe' (14). The project started in June 2005 and came to an end in December 2009. The project partners are primarily based in Europe, but there are also partners from Russia (Siberia), India and China (together called 'INCO partners'), giving the project a wide variation in cultures, eating habits and environments.

One part of EuroPrevall was designed to estimate the currently unknown prevalence of food allergy and exposure to known or suspected risk factors for food allergy across European children in the age of 6–12 years. A detailed overview of the background and study methodology has been published earlier (15). In summary, the surveys were performed as multicentre, cross-sectional studies in general child populations. Case-control studies were nested within these surveys. These studies were undertaken in eight European countries and in the INCO countries India, China and Russia (16). Participating study centres in this study were Hong Kong (China), urban and rural Tomsk (Russia), Mysore and Bangalore (India), Athens (Greece) and Utrecht (the Netherlands). In

rural Tomsk Oblast, areas were included where a fish parasitic helminth infection (*Opisthorchis felinus*) is prevalent. Greece and the Netherlands were selected from the European partners as they were expected to be countries with a low and high prevalence of food allergy, respectively. After a community-based survey collecting basic information on adverse reactions to foods, all children stating they had experienced such reactions, as well as a random sample of those stating 'no reactions' to foods, completed a detailed questionnaire on potential risks and exposures. A blood sample was taken for serological analysis of food and aeroallergen sensitization. In INCO countries, skin prick testing (SPT) in all children was also performed (16).

In a subsample of the sera from the children with complete questionnaire data, antibodies against foodborne and orofecal pathogens were determined. Within the Greek, Dutch and Russian centres, a random selection of 80% of the identified cases was made. Where possible, an equivalent number of controls were then randomly selected for each centre. Within Hong Kong, there were relatively fewer potential controls compared with cases, and a random selection of 100 cases and 60 controls was made. For India, data were available for 194 children (124 from Bangalore and 70 from Mysore), of which only 33 of them were identified as case. Furthermore, blood samples from most of the cases were lost during transport to the central laboratory, and infection serology could not be measured. For these reasons, all Indian children were excluded from this study.

In total, 898 children participated from five study centres: 127 from Athens, 249 from Utrecht, 158 from Hong Kong, 129 from urban Tomsk and 235 from rural Tomsk (Table 1). The study was approved by the local ethics committees of all participating centres. Parents or guardians of all children gave informed consent.

Table 1 Selection of cases and controls for measurement of infection serology of all cases and controls per study centre

Study centre	Cases			Controls		
	N	Mean age	% Male	N	Mean age	% Male
Athens						
Selected	58	9.8	56.9	69	9.8	46.4
Not selected	13	10.3	53.8	64	9.8	43.8
Utrecht						
Selected	137	9.5	57.7	112	9.4	56.3
Not selected	31	9.7	41.9	143	9.4	54.9
Hong Kong						
Selected	97	9.2	59.8	61	9.1	41.0
Not selected	177	9.3	58.2	66	8.9	37.9
Urban Tomsk						
Selected	64	9.5	60.9	65	9.6	53.8
Not selected	17	9.5	70.6	273	9.5	42.9
Rural Tomsk						
Selected	99	10.0	47.5	136	10.0	47.8
Not selected	24	10.0	62.5	610	9.9	48.0

Definition cases and controls

For this analysis, cases and controls were defined by allergic sensitization to any allergen, and controls also reported no wheezing or whistling in the chest for the past 12 months. It is known that the agreement between SPT and specific IgE is better in affluent countries compared with developing countries. In developing countries, there is a large discrepancy between sIgE and SPT. While children in Ghana can have IgE to peanut (17.5%), the SPT response is only 2.0% (17). Based on this, the study design aimed to look at SPT and not sIgE when it comes to INCO countries. Therefore, allergic sensitization was based on SPT in INCO but sIgE (as no SPT was performed) in European centres.

Serum IgE

Using an ImmunoCAP 250 system (Thermo Fisher Scientific, Uppsala, Sweden), specific IgE was determined for the following aeroallergens: house dust mite, cat, grass, birch, *Parietaria* pollen and mugwort; and for the following food allergens: hen's egg, cow's milk, soy, peanut, wheat, fish, sesame seed, buckwheat, corn, hazelnut, tomato, walnut, carrot, celery, shrimp, poppy seed, lentil, mustard, sunflower, apple, kiwi, melon, banana, peach and additionally relevant allergens in China (mango and crab). A serum-specific IgE value of ≥ 0.35 kU_A/l was considered positive (16). IgE sensitization to food, aeroallergens or any allergen was defined by positive serology to at least one of the food, aero or any allergen(s), respectively.

Skin prick testing

Nine common aeroallergens (dustmite mix, cockroach, cat, birch pollen, grass pollen mix, mugwort pollen, ragweed pollen, chenopodium pollen and date palm profilin) and 27 food allergens (peanut, cow's milk, hen's egg, soy, fish, shrimp, mussel, hazelnut, walnut, melon, wheat, lentils, chickpea, egg plant, banana, sunflower, tomato, jackfruit, carrot, beef, orange, apple (non-birch-pollen and birch-pollen-related apple allergen), crab, mango, peach and mustard) were used for skin testing. All allergens were obtained from ALK-Abelló (Madrid, Spain) (15).

Skin testing was performed according to EAACI guidelines (18). Histamine was used as the positive control and diluent as the negative control. Skin prick tests were considered positive if the mean wheal diameter was equal to or greater than 3 mm. SPT sensitization to food, aeroallergens or any allergen was defined as having at least one positive SPT to food, aeroallergen or any allergen, respectively. SPT to peach and mango was performed only in half of the children (55 of 110) in Hong Kong. All measured children were negative to these two food allergens; therefore, peach and mango were not included in the positive SPT to any (food) allergen.

Antibodies against foodborne and orofecal pathogens

As marker of previous exposure to hepatitis A, *T. gondii* and salmonella, the presence of antibodies against these pathogens

was determined in serum samples. Total antibodies to hepatitis A were tested by means of a competitive enzyme immunoassay (ETI-AB-HAVK PLUS, DiaSorin, Italy) and IgG antibodies to *T. gondii* and salmonella were tested using immunoenzymatic assays (ETI-TOXOK-G PLUS, DiaSorin, Italy and Salmonella Typhi IgG ELISA, Calbiotech Inc., San Diego, CA, USA, respectively). Results were expressed as positive or negative, using the following cut-offs for positivity: ≤ 1.0 OD sample/cut-off ratio for hepatitis A (with cut-off value of 20 mIU/ml as given by manufacturer), >13.5 IU/ml for *T. gondii* and >1.1 OD sample/cut-off ratio for salmonella. The infection serology was repeated for 70 children, and for them the mean value was used to define exposure to the pathogens.

Statistical analyses

Within study centre and case/control group, the selected children were compared with the unselected children for age and sex by independent samples *t*-test and chi-square test, respectively. For each study centre, the associations between allergic sensitization (to food, aero and any allergen(s)) and antibodies to the three pathogens were analysed by contingency tables, chi-square tests and logistic regression (Table S1). Testing for heterogeneity of odds ratios across study centres (using interaction terms) showed no significant heterogeneity for each association. We therefore performed the analyses on all children from the study centres together, using logistic regression analyses with adjustment for study centre. Possible confounding was then analysed using the change-in-estimate criterion of 10%. When a variable changed the odds ratio (OR) with more than 10%, this variable was left in the model, regardless of significance (19). Potential confounders were sex, age, presence of older siblings, SES (based on education of the mother), parental atopy (food allergy, asthma, eczema and/or hay fever), parental smoking in the household (currently and in first 2 years of child's life), antibiotic use in first 2 years, day care attendance (attendance of playschool or nursery with older children before age of 2 years), overcrowding (>2 children per sleeping room before age of 2 years), breastfeeding (>1 month), and current and past animal exposure (cat, dog, horse, bird, guinea pig; in first 2 years also rabbit and mouse). The variables above were also explored for effect modification using interaction terms. Results are reported as odds ratios with 95% confidence intervals (95% CI) and *p*-values. For statistical analyses, we used SPSS (SPSS Inc., Chicago, IL, USA) for Windows version 17. *P*-values below 0.05 were considered statistically significant.

Results

Study population

From Athens, Utrecht, Hong Kong, urban and rural Toms, 898 children were randomly selected for this study. Within study centre and case/control group, no significant differences were found in the distribution of age and gender for those selected compared with those not selected (Table 1).

Three controls (from Athens, Utrecht and urban Tomsk) were excluded from the analyses because they reported wheezing. Of the 521 children from the INCO countries, 95 children had a negative SPT to histamine (48 from Hong Kong, 9 from urban Tomsk and 38 from rural Tomsk). No major differences were found between children with negative and positive histamine reactions, with the exception that histamine positives were more likely to have positive SPT responses (OR = 2.33, $p = 0.001$ for aeroallergens; OR = 2.39, $p < 0.001$ for any allergen, with adjustment for study centre). Therefore, the 95 histamine negatives were excluded, and 800 children remained for analysis. The characteristics of this study population and the distribution of possible confounders are shown in Table 2. A total of 380 children were defined as controls and 420 children as cases. The age range of the children was 6–12 years (median 9.5 years). Allergic sensitization was positively associated with study centre, maternal education, parental atopy and antibiotic use, and negatively associated with past and current parental smoking and current animal exposure ($p < 0.05$). Table S2 shows the number and percentage of SPT and sIgE sensitization to the specific allergens per study centre.

Exposure to pathogens

Table 3 shows the number and percentage of positive serology to each of the three pathogens per study centre and case/control status. In this study, we found that from all children in the 5 study centres, 25.6% was exposed to salmonella, 7.5% to

T. gondii, 18.0% to hepatitis A and 43.8% to at least one of the three pathogens. The percentage of seropositivity to salmonella is the highest in Hong Kong (46.4%), whereas hepatitis A seropositivity is very high in Athens (71.2%). *T. gondii* seropositivity generally has the lowest percentage and is the most common in urban Tomsk (13.4%).

Pathogen exposure and allergic sensitization

Table 4 shows the associations between exposure to each pathogen and the three outcomes (sensitization to any allergen, any food allergen, any aeroallergen), based on analyses of all children. Although all associations show no statistical significance, *T. gondii* tends to have a negative association with 'any sensitization' (OR = 0.62, $p = 0.092$) and hepatitis A tends to have a positive association with food and any sensitization (OR = 1.58, $p = 0.065$ and OR = 1.70, $p = 0.053$, respectively). No differences were found for exposure to any of the three pathogens between sensitized and non-sensitized children (Table 4). After adjusting for study centre, no confounding variables were found.

Discussion

We investigated the association between markers of exposure to three different foodborne and orofecal pathogens with allergic sensitization to food and aeroallergens among school-aged children in Utrecht, Athens, Hong Kong and urban and rural Tomsk. Although not significant, the

Table 2 Characteristics and distribution of possible confounders in cases and controls

Variables	All (N = 800) n (%)	Controls (N = 380) n (%)	Cases (N = 420) n (%)	OR	p
Study centre					
Athens	126 (15.8)	68 (17.9)	58 (13.8)	Ref	
Utrecht	248 (31.0)	111 (29.2)	137 (32.6)	1.45	0.093
Hong Kong	110 (13.8)	36 (9.5)	74 (17.6)	2.41	0.001
Urban Tomsk	119 (14.9)	60 (15.8)	59 (14.0)	1.15	0.578
Rural Tomsk	197 (24.6)	105 (27.6)	92 (21.9)	1.03	0.906
Maternal education					
No/primary	83 (10.4)	46 (12.1)	37 (8.8)	Ref	
Secondary	306 (38.3)	153 (40.4)	153 (36.5)	1.24	0.381
Lower level vocational	76 (9.5)	33 (8.7)	43 (10.3)	1.62	0.132
Higher level vocational	85 (10.7)	42 (11.1)	43 (10.3)	1.27	0.436
University	248 (31.1)	105 (27.7)	143 (34.1)	1.69	0.039
Girl	370 (46.3)	188 (49.5)	182 (43.3)	0.78	0.082
Older siblings	361 (45.4)	177 (46.7)	184 (44.1)	0.90	0.466
Parental atopy	525 (68.8)	220 (61.6)	305 (75.1)	1.88	<0.001
Past parental smoking	310 (38.9)	172 (45.6)	138 (32.9)	0.59	<0.001
Current parental smoking	271 (34.0)	151 (39.8)	120 (28.6)	0.61	0.001
Antibiotics	408 (51.3)	162 (43.0)	246 (58.9)	1.90	<0.001
Day care	258 (32.3)	130 (34.3)	128 (30.5)	0.84	0.258
Overcrowding	30 (3.8)	19 (5.0)	11 (2.6)	0.51	0.080
Breastfeeding	570 (71.4)	278 (73.4)	292 (69.7)	0.84	0.253
Past animal exposure	340 (42.6)	169 (44.6)	171 (40.8)	0.86	0.281
Current animal exposure	407 (51.0)	211 (55.7)	196 (46.8)	0.70	0.012

Statistically significant results are shown in bold.

Table 3 Seropositivity to the measured pathogens per study centre and case/control status

	Athens N = 126 n (%)	Utrecht N = 248 n (%)	Hong Kong N = 110 n (%)	Urban Tomsk N = 119 n (%)	Rural Tomsk N = 197 n (%)	All N = 800 n (%)
Positive serology to						
Salmonella						
All	29 (23.2)	21 (8.5)	51 (46.4)	28 (25.2)	67 (38.7)	196 (25.6)
Controls	14 (20.9)	9 (8.1)	21 (58.3)	16 (27.1)	36 (35.3)	96 (25.6)
Cases	15 (25.9)	12 (8.8)	30 (40.5)	12 (23.1)	31 (43.7)	100 (25.6)
<i>T. gondii</i>						
All	4 (3.2)	18 (7.3)	5 (4.5)	15 (13.4)	16 (8.9)	58 (7.5)
Controls	1 (1.5)	10 (9.0)	3 (8.3)	10 (16.9)	11 (10.8)	35 (9.3)
Cases	3 (5.2)	8 (5.9)	2 (2.7)	5 (9.4)	5 (6.5)	23 (5.8)
Hepatitis A						
All	89 (71.2)	32 (13.0)	10 (9.1)	5 (4.5)	3 (1.7)	139 (18.0)
Controls	43 (64.2)	12 (10.8)	5 (13.9)	1 (1.7)	1 (1.0)	62 (16.2)
Cases	46 (79.3)	20 (14.7)	5 (6.8)	4 (7.5)	2 (2.6)	77 (19.3)
Any pathogen						
All	95 (76.0)	65 (26.2)	56 (50.9)	42 (37.5)	78 (45.1)	336 (43.8)
Controls	46 (68.7)	26 (23.4)	23 (63.9)	23 (39.0)	44 (43.1)	162 (43.2)
Cases	49 (84.5)	39 (28.7)	33 (44.6)	19 (35.8)	34 (47.9)	174 (44.4)

Table 4 Association between exposure to pathogens and sensitization to any allergen, any food allergen and any aeroallergen. Models are adjusted for study centre.

	Pathogen	OR	95% CI	p
Any sensitization	Salmonella	1.00	0.71–1.42	0.992
	<i>T. gondii</i>	0.62	0.36–1.08	0.092
	Hepatitis A	1.58	0.97–2.58	0.065
	Any pathogen	1.12	0.82–1.52	0.473
Food sensitization	Salmonella	0.98	0.62–1.53	0.916
	<i>T. gondii</i>	0.64	0.30–1.34	0.237
	Hepatitis A	1.70	0.99–2.90	0.053
	Any pathogen	1.12	0.77–1.63	0.563
Aero sensitization	Salmonella	0.99	0.70–1.41	0.952
	<i>T. gondii</i>	0.69	0.39–1.21	0.195
	Hepatitis A	1.42	0.87–2.31	0.156
	Any pathogen	1.15	0.84–1.57	0.383

OR, odds ratios; 95% CI, 95% confidence intervals.

association between *T. gondii* and hepatitis A seropositivity with allergic sensitization tends to be negative and positive, respectively.

Epidemiological studies have found negative associations of *T. gondii* with asthma and hay fever (7), with IgE sensitization (6, 8, 10, 20), and with SPT (10) to aeroallergens. Although we found similar effect sizes in our study, this was not statistically significant.

To our knowledge, this is the first time that a positive trend is reported between exposure to hepatitis A and sensitization to food and aeroallergens. Consistent with our finding that children with a positive serology to hepatitis A tend to be more sensitized to aeroallergens, von Hertzen et al. (21) reported a similar (but non-significant) positive association between hepatitis A and SPT to aeroallergens in Russian adults

(OR = 1.92, $p = 0.087$). However, there are also a number of studies that have reported a negative association (6, 7) or no association (9, 10, 22, 23) between hepatitis A and SPT or sIgE sensitization.

The percentage of hepatitis A seropositivity was highest in Athens, namely 71.2%, compared with 1.7–13.0% of the other study centres. To assess whether Athens could have a disproportionate influence on the results, we reanalysed the data for only Utrecht, Tomsk and Hong Kong but found the same direction for the association of hepatitis A with sensitization to allergens (any allergen: OR = 1.33, $p = 0.363$; any food allergen: OR = 1.47, $p = 0.248$; any aeroallergen: OR = 1.36, $p = 0.317$).

The inconsistent results between different studies might be explained by interference from vaccination to hepatitis A, because the immunological effects from vaccination and natural exposure may be different. There are even studies that have shown that vaccinated individuals may be more prone to allergic diseases (24, 25). Unfortunately, we do not have vaccination data for this study population, so we are unable to investigate this further.

It is the first time that the association between salmonella and sensitization was investigated with a general serology to estimate exposure to salmonella at the population level. We found that exposure to salmonella appeared not to have an association with sensitization to food or aeroallergens. An earlier study investigating salmonellosis in infancy and respiratory allergies at the age of 6–18 years found a protective effect on respiratory allergies (13). However, that study examined children hospitalized with salmonella infection, which is entirely different from our population-based study. These findings suggest that clinical infection and not just exposure to salmonella induces tolerance to allergens.

Exposure to any of the three measured pathogens was not associated with allergic sensitization. This finding is probably

due to different percentages of seropositivity in the different study centres and the opposite odds ratios for *T. gondii* and hepatitis A (Table S1).

A limitation of our study is that we do not know whether the pathogen exposure came before or after the sensitization, as we are not able to distinguish the timing of exposure. The orofecal pathogens that we studied usually occur during school age years, when the immune system is already activated and possible sensitizations could already exist. Furthermore, besides no sensitization to any allergen, we selected controls to have no wheezing in the past 12 months. This means that we selected a control group that may have been poorly representative of unsensitized children. To study this potential flaw in the design, we analysed only children without wheezing (109 cases with wheezing were excluded). The same directions of all associations and a significant negative association between *T. gondii* and sensitization to any allergen were found (data not shown).

A strength of this study is that, to the best of our knowledge, it is the first study to investigate the association between these three pathogens and allergic sensitization in such a geographically diverse setting. In addition, the same standardized methodology and laboratory measurements across all countries were used, allowing valid comparisons to be made across countries and regions.

Exposure status was defined as being positive or negative, using binary cut-offs, thereby maybe losing information. However, when we used exposure to salmonella and *T. gondii* in three groups (positive, negative and borderline), the same results were found (data not shown).

In conclusion, in this study of school-aged children from different countries, a tendency was found for a negative association between *T. gondii* seropositivity and for a positive association between hepatitis A seropositivity and allergic sensitization to food and aeroallergens. Further studies with larger sample size taking into account the symptoms of food

allergy are needed to clarify the relationship of food allergies and past foodborne and orofecal exposure.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

JJJ – carried out the statistical analysis and drafted the paper; GWKW – conducted the study in Hong Kong; JP – cleaned and prepared the database; LMO – supervised the study in Tomsk; OSF – conducted the study in Tomsk; PAM – conducted the study in India; AS – conducted the study in Athens; NGP – supervised the study in Athens; ACK – conducted the study in Utrecht; SAV – measured IgE antibodies; ACMK – involved in discussion of the results; ACTMV – supervised measurements of exposure to pathogens; MCP – supervised the statistical analysis and reviewed the first draft; IK – supervised the INCO and other epidemiological studies in EuroPrevall and reviewed the text; PB – supervised data collection and epidemiological design, advised on statistical analysis and reviewed the manuscript; RvR – supervised the study, advised on allergic parameters, reviewed the manuscript; MY – supervised the study, the data analysis and reviewed the manuscript. All authors have read and approved the final manuscript.

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Supporting Information

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

Table S1. Associations between exposure to pathogens and sensitization to any allergen, any food allergen, and any aeroallergen per study centre.

Table S2. Allergic sensitization to all separate allergens, determined by positive sIgE in Athens (N = 126) and Utrecht (N = 248), and by positive SPT in Hong Kong (N = 110), urban Tomsk (N = 119) and rural Tomsk (N = 197).

Table S1 Associations between exposure to pathogens and sensitisation to any allergen, any food allergen, and any aeroallergen per country, using sIgE in Europe and SPT in INCO-countries. Athens N=126, Utrecht N=248, Hong Kong N=110, urban Tomsk N=119 and rural Tomsk N=197

Pathogen	Study centre	Any allergen			Any food allergen			Any aeroallergen		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Salmonella	Athens	1.32	0.58 – 3.04	0.512	1.61	0.64 – 4.05	0.314	0.84	0.35 – 2.00	0.693
	Utrecht	1.10	0.45 – 2.71	0.841	0.48	0.16 – 1.49	0.205	1.46	0.59 – 3.59	0.414
	Hong Kong	0.49	0.22 – 1.09	0.081	0.99	0.31 – 3.16	0.987	0.49	0.22 – 1.09	0.081
	Urban Tomsk	0.81	0.34 – 1.91	0.625	0.84	0.28 – 2.55	0.763	0.77	0.32 – 1.83	0.550
	Rural Tomsk	1.42	0.76 – 2.64	0.267	1.15	0.48 – 2.77	0.750	1.58	0.84 – 2.95	0.155
<i>T. gondii</i>	Athens	3.60*	0.36 – 35.6	0.273	3.32*	0.45 – 24.7	0.241	5.25*	0.53 – 52.0	0.156
	Utrecht	0.63	0.24 – 1.66	0.350	0.82	0.28 – 2.39	0.719	0.65	0.25 – 1.75	0.396
	Hong Kong	0.31*	0.05 – 1.92	0.206	-	-	-	0.31*	0.05 – 1.92	0.206
	Urban Tomsk	0.51	0.16 – 1.60	0.250	0.59	0.12 – 2.84	0.513	0.58	0.18 – 1.82	0.348
	Rural Tomsk	0.57	0.19 – 1.73	0.324	-	-	-	0.67	0.22 – 2.01	0.473
Hepatitis A	Athens	2.14	0.95 – 4.80	0.065	2.42	0.85 – 6.93	0.099	1.54	0.68 – 3.53	0.303
	Utrecht	1.42	0.66 – 3.06	0.366	2.13	1.00 – 4.52	0.049	1.42	0.67 – 3.01	0.354
	Hong Kong	0.45	0.12 – 1.67	0.231	-	-	-	0.45	0.12 – 1.67	0.231
	Urban Tomsk	4.74*	0.51 – 43.8	0.171	1.02*	0.11 – 9.64	0.984	5.30*	0.57 – 49.1	0.142
	Rural Tomsk	2.69*	0.24 – 30.3	0.422	-	-	-	3.10*	0.28 – 34.9	0.359
Any pathogen	Athens	2.49	1.03 – 5.98	0.042	1.79	0.62 – 5.17	0.285	1.92	0.77 – 4.74	0.160
	Utrecht	1.31	0.74 – 2.34	0.352	1.27	0.70 – 2.30	0.442	1.45	0.82 – 2.56	0.202
	Hong Kong	0.46	0.20 – 1.03	0.060	0.81	0.25 – 2.57	0.715	0.46	0.20 – 1.03	0.060
	Urban Tomsk	0.88	0.41 – 1.88	0.732	0.94	0.36 – 2.48	0.902	0.89	0.41 – 1.93	0.768
	Rural Tomsk	1.21	0.66 – 2.23	0.537	0.85	0.36 – 2.04	0.717	1.38	0.74 – 2.55	0.308

OR: odds ratios, 95% CI: 95% confidence intervals

*: Unstable OR due to low numbers in cells

-: OR cannot be calculated

Statistically significant results are shown in bold.

Table S2 Allergic sensitisation to all separate allergens, determined by positive sIgE in Athens (N=126) and Utrecht (N=248), and by positive SPT in Hong Kong (N=110), urban Tomsok (N=119) and rural Tomsok (N=197)

	Athens		Utrecht		Hong Kong		Urban Tomsok		Rural Tomsok	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Hen's egg	5	(4.0)	14	(5.6)	1	(0.9)	5	(4.2)	4	(2.0)
Cow's milk	20	(15.9)	27	(10.9)	1	(0.9)	1	(0.8)	2	(1.0)
Soy	5	(4.0)	17	(6.9)	0	(0.0)	4	(3.4)	3	(1.5)
Peanut	6	(4.8)	25	(10.1)	1	(0.9)	12	(10.1)	7	(3.6)
Wheat	9	(7.1)	30	(12.1)	0	(0.0)	1	(0.8)	1	(0.5)
Fish	0	(0.0)	3	(1.2)	3	(2.7)	6	(5.0)	13	(6.6)
Hazelnut	5	(4.0)	40	(16.1)	1	(0.9)	12	(10.1)	6	(3.0)
Tomato	6	(4.8)	21	(8.5)	0	(0.0)	3	(2.5)	1	(0.5)
Walnut	8	(6.3)	13	(5.2)	0	(0.0)	-		-	
Carrot	7	(5.6)	27	(10.9)	-		8	(6.7)	8	(4.1)
Shrimp	2	(1.6)	10	(4.0)	8	(7.3)	-		-	
Sunflower	7	(5.6)	17	(6.9)	-		3	(2.5)	2	(1.0)
Apple	7	(5.6)	29	(11.7)	0	(0.0)	3	(2.5)	2	(1.0)
Melon	5	(4.0)	15	(6.0)	0	(0.0)	2	(1.7)	2	(1.0)
Peach	9	(7.1)	33	(13.3)	0	(0.0)	-		-	
Crab	-		-		4	(3.6)	-		-	
Mango	-		-		0	(0.0)	-		-	
Banana	12	(9.5)	30	(12.1)	-		-		-	
Sesame seed	9	(7.1)	23	(9.3)	-		-		-	
Buckwheat	8	(6.3)	16	(6.5)	-		-		-	
Corn	8	(6.3)	21	(8.5)	-		-		-	
Celery	5	(4.0)	27	(10.9)	-		-		-	
Poppy seed	4	(3.2)	17	(6.9)	-		-		-	
Lentil	5	(4.0)	18	(7.3)	-		-		-	
Mustard	2	(1.6)	7	(2.8)	-		-		-	
Kiwi	6	(4.8)	36	(14.5)	-		-		-	
Mussel	-		-		0	(0.0)	-		-	
Beef	-		-		0	(0.0)	-		-	
Orange	-		-		0	(0.0)	-		-	
Apple (birch-related)	-		-		-		9	(7.6)	6	(3.0)
Any food	30	(23.8)	79	(31.9)	13	(11.8)	28	(23.5)	36	(18.3)
HDM	19	(15.1)	88	(35.5)	74	(67.3)	21	(17.6)	51	(25.9)
Cat	17	(13.5)	38	(15.3)	11	(10.0)	23	(19.3)	24	(12.2)
Grass	26	(20.6)	72	(29.0)	0	(0.0)	12	(10.1)	19	(9.6)
Birch	6	(4.8)	48	(19.4)	-		27	(22.7)	25	(12.7)
Mugwort	14	(11.1)	24	(9.7)	0	(0.0)	9	(7.6)	18	(9.1)
Parietaria	21	(16.7)	20	(8.1)	-		-		-	
Cockroach	-		-		3	(2.7)	3	(2.5)	6	(3.0)
Chenopodium	-		-		-		10	(8.4)	7	(3.6)
Ragweed	-		-		-		7	(5.9)	4	(2.0)
Date palm	-		-		0	(0.0)	1	(0.8)	3	(1.5)
Any aero	47	(37.3)	120	(48.4)	74	(67.3)	56	(47.1)	83	(42.1)
Any allergen	58	(46.0)	137	(55.2)	74	(67.3)	59	(49.6)	92	(46.7)

- Allergen not relevant and not measured in this country, or allergen only measured with sIgE or SPT