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Incidence and natural history of hen's egg allergy in the first 2 years of life – the EuroPrevall birth cohort study

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Short title: Egg allergy in -the EuroPrevall birth cohort

Abbreviations

HE	Hen's egg
HEA	Hen's egg allergy
DBPCFC	Double-blind, Placebo-Controlled Food Challenges
SPT	Skin prick testing
IgE	Immunoglobulin E
CI	Confidence Interval
SCORAD	SCOR ing A topic D ermatitis

Abstract

Background: Parents and health staff perceive hen's egg allergy (HEA) as a common food allergy in early childhood but the true incidence is unclear because population-based studies with gold-standard diagnostic criteria are lacking.

Objective: To establish the incidence and course of challenge-confirmed HEA in children, from birth until the age of 24 months, in different European regions.

Methods: In the EuroPrevall birth cohort study, children with a suspected HEA and their age-matched controls were evaluated in 9 countries, using a standardized protocol including measurement of HE-specific immunoglobulin E-antibodies in serum, skin prick tests and double-blind, placebo-controlled food challenges (DBPCFC).

Results: Across Europe 12,049 newborns were enrolled, 9336 (77.5%) were followed up to 2 years of age. In 298 children, HEA was suspected and DBPCFC was offered. HEA by age two was confirmed in 86 out of 172 challenged children (mean raw incidence 0.84%, 95% confidence interval (95% CI) 0.67-1.03). Adjusted mean incidence of HEA was 1.23% (95% CI 0.98-1.51) considering possible cases among eligible children who were not challenged. Centre-specific incidence ranged from United Kingdom (2.18%, 95% CI 1.27-3.47) to Greece (0.07%). Half of the HE allergic children became tolerant to HE within one year after the initial diagnosis.

Conclusions: The largest multinational European birth cohort study on food allergy with gold-standard diagnostic methods showed that the mean adjusted incidence of HEA was considerably lower than previously documented, although differences in prevalence rates

among countries were noted. Half of the children with documented HEA gained tolerance within one year post-diagnosis.

Key words:

Epidemiology, Egg Allergy, Children,

Introduction

Hen's egg (HE) is a common food source in many cultures, while egg protein is included in a wide range of cooked and manufactured foods. HE allergy (HEA) is considered an increasingly serious health issue in children both by parents and health care professionals and like other allergies can be life threatening (1). Studies in Western countries have indicated that HE represents the second most prevalent allergen during infancy and early childhood following cow's milk (2-4), whereas studies from Japan and China reported that HEA was the most frequently recognized food allergy (5,6).

To date, the only European - wide study including data on HEA is a telephone survey using a standardized questionnaire administered to 44,000 people across 10 countries. According to parents' perception, HE represented the third causative allergenic agent, following milk and fruits, except for Slovenia and Greece where HE was the first perceived food allergen in 2 to 3-year olds (7). The proportion of affected children who "outgrow" HEA and its timing is unclear (8,9).

The aim of this manuscript is to assess the incidence and natural course of HEA up to the age of 24 months across Europe using data from the birth cohort of the EuroPrevall project.

Materials and methods

Study population

The EuroPrevall birth cohort study was a longitudinal prospective study, including children recruited from nine countries across Europe, between October 2005 and March 2007. Study design, methods and baseline characteristics of the study participants have been described previously (10-12). Written informed consent was obtained from parents and evaluation commenced at birth and included two follow-up assessments of all participants at 12 and 24 months regardless of allergy status. Study centers invited all eligible pregnant women to participate in the study over an average period of 1.5 years.

Study design

Baseline and follow-up questionnaires

At recruitment, standardized questionnaires that included information on socio-demographic characteristics, environmental exposures and family history of allergy were administered to parents. Subsequently, trained personnel performed telephone questionnaires at age 12 and 24 months, and collected data on breastfeeding, weaning practices, the child's food intake, and any signs and/or symptoms suggestive of allergic diseases such as eczema or an adverse food reaction. Parental reporting was encouraged by intermittent reminders either by telephone, e-mail or text messages.

Evaluating children with suspected hen's egg allergy.

Children with eczema or a suspected adverse reaction to HE, either reported by parents to the study center or identified within the standardized telephone screening assessments, were invited for standardized clinical approach consisting of physical examination and eczema symptom scoring with the Scoring Atopic Dermatitis (SCORAD) scale, venous blood sampling and skin prick testing (SPT) (ALK-Abelló, Hørsholm, Denmark) (13). Allergic sensitization to HE was assessed either by SPT or by determination of serum specific Immunoglobulin E (IgE) antibodies to HE (Phadia ImmunoCap 250 system, Thermo Fisher, Uppsala, Sweden) carried out in a central laboratory (Charité - Universitätsmedizin Berlin, Berlin, Germany).

A child was eligible for HE double-blind, placebo-controlled food challenge test (DBPCFC) if at least one of the following criteria was fulfilled: a) HE specific IgE ≥ 0.35 KU/L and/or SPT's wheal diameter ≥ 3 mm (unless the child consumed HE without clinical signs or symptoms), b) objective signs/symptoms within two hours of HE ingestion or c) repetitive parent-reported symptoms after HE ingestion that improved following HE elimination diet.

Re-assessment of confirmed food allergic children was scheduled 12-months after diagnosis.

Double-Blind, Placebo-Controlled Food Challenges (DBPCFC)

DBPCFC were conducted in study centers under the supervision of a trained pediatrician using a standardized protocol and recipe (10). We performed active and placebo arms of the challenge tests on separate days in a random order at least 48 hours apart. The active challenge contained pasteurized egg powder (Dairy Crest Group, Esher, United Kingdom and

Vreughdenhil, Netherlands). DBPCFC consisted of nine steps, with increasing doses of egg powder every 20-30 minutes. All necessary precautions and emergency equipment were available in the event of severe adverse reaction (adrenaline, nebulized salbutamol, hydrocortisone, chlorphenamine, fluid resuscitation). The challenge was stopped upon presentation of pre-defined symptoms and unblinded after completion of the last challenge day. A positive food challenge was based on objective immediate reactions, such as urticaria, angioedema, flush, vomiting, diarrhoea, respiratory symptoms, and immediate worsening of eczema with an objective SCORAD score increase ≥ 10 , which led to abortion of the challenge procedure or which appeared not later than 2 hours after the final dose. Additionally, worsening of eczema within 48 hours after starting the challenge was also rated as reactive when supported by a documented increase of SCORAD of ≥ 10 , which is greater than the minimally clinically important difference (MICD) (13). DBPCFC were not performed in children with a history of severe anaphylactic reaction to HE; however, these children were labeled as cases and included in the current analysis.

Sample size and statistical analysis

Sample size estimation has been described previously (10). We conducted statistical analysis for each country separately and then combined for the total cohort. All children with valid date of birth and sex were included in the analysis. Educational level was coded in three broad categories (high, moderate, low), embracing inter-country differences of educational systems. Allergy status of parents was based on self-reported doctor's diagnoses of asthma, allergic rhinitis, eczema and/or food allergy.

Missing items did not lead to the exclusion of cases and/or follow-up data. Time at risk and under observation was calculated as the age at first diagnosis or for non-cases age at last documented follow-up plus 6 months, assuming parents would have reported suspicious symptoms even after the last contact to the study centre.

2-year-incidence was calculated from incidence rates. We estimated the probability for a positive food challenge by age using logistic regression for each centre separately. Adjusted estimates of the incidence were based on adjustments for possible cases among children who were (i) lost to follow-up; (ii) eligible but not challenged, assuming the same distribution of challenge outcomes as in those who were challenged; (iii) placebo reactors or

had inconclusive challenge outcomes (all by centre). All statistical computations were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Participants

From October 2005 to January 2010, 12,049 babies and their families from the nine participating European centers were included in the study (10). By the age of 2 years, 2,713 children (22.5%) dropped out from follow up (figure 1). No differences were noted in respect to baseline characteristics of loss to follow-up families compared to recruited children (Table 1).

Investigation and characteristics of children with suspected HEA.

2,612 children were identified as potentially having HEA, either by parental report or during annual follow up questionnaires (1,928 and 684 respectively). These children were assessed by telephone interview and 1,117 cases were subsequently invited to visit their respective centers. Following a standardized assessment, 298 (27%) children were defined as eligible for DBPCFC. 4 more children without documented eligibility criteria were challenged (total challenged 172). In a considerable proportion of eligible children (42%, n=126) a DBPCFC was not performed, either due to parental refusal or a history of anaphylactic reaction to HE (Figure 1).

No major differences were noted in regards to baseline characteristics in challenged compared to not-challenged children; nevertheless parents with a history of allergy, of male, younger and children with eczema, positive HE specific IgE or SPT were more likely to consent for DBPCFC (table 1).

Mean age at eligibility was 12 months, while mean time interval between assessment at the study center and DBPCFC was 4 months.

Fifty percent (n=86) out of 172 children (mean age 17 months) had a positive DBPCFC for HEA. The highest number of DBPCFC (n=42) was performed in Iceland (table 2). The vast majority of HE allergic children were sensitized to HE reflected either by a positive SPT (64%) and/or positive serum IgE test to HE (79.1%) (table 1). DBPCF included in the present analysis met all quality criteria defined by the study protocol including the start of the last dose on both arms of the challenge tests.

Incidence of HEA by the age of two years

The overall raw incidence of HEA by the age of two years was 0.84% (95%CI 0.67-1.03) (table 2). Rates in individual countries varied from 0.11% in Greece to 1.95% in UK. Mediterranean (Spain, Italy, and Greece) and Eastern European countries (Lithuania, Poland) displayed lower HEA incidences than North-Western countries.

Following adjustment for possible cases among children who were (i) eligible but not challenged, (ii) placebo reactors or had inconclusive challenge outcomes, or (iii) lost-to-follow-up (all by centre), HEA mean incidence was estimated at 1.23% (95%CI 0.98-1.51%) (figure 2). When stratified by center, United Kingdom reported the highest incidence for HEA (2.18%; 95%CI 1.27-3.47) while Greece had the lowest incidence (0.07%; 95%CI 0.00%-0.37%).

Mean age for initial HEA diagnosis was 17 months, with variations recorded among centers (lowest in Netherlands: 13 months, highest in Poland: 25 months). Half (50%) of children suspected of having HEA at initial screening had a negative DBPCFC.

Symptoms in positive HE DBPCFC's

Symptoms in positive DBPCFC were mainly of the immediate type, occurring within two hours following the highest tolerated dose administered. These included primarily skin manifestations, such as urticaria, flushing, angioedema, and eczema deterioration (table 3).

A high proportion of children presented with gastrointestinal symptoms, mainly vomiting, followed by symptoms from the upper and lower airways. Delayed reactions (>2h post-DBPCFC) were reported only in around 20% of all positive challenge tests: urticaria and eczema deterioration. A history of severe anaphylactic reactions after HE consumption led to two children being categorized as HEA without performing DBPCFC.

Age at introduction of hen's egg and development of hen's egg allergy

In our cohort, HE was introduced into the diet in nearly 80% of all children by their first birthday, while around 90% of all participants had consumed HE by the age of 15 months. The cumulative incidence of HE consumption and HEA continued to run in parallel up to the age of two years (figure 3).

Development of HE tolerance

Reassessment of all children with DBPCFC-proven HEA was scheduled one year after their positive food challenge test (table 2). Twenty-four out of 49 re-evaluated children (49%) had become tolerant to HE as assessed by a follow-up DBPCFC one year post initial diagnosis.

Discussion

Key findings

The EuroPrevall birth cohort is the largest longitudinal observational population-based study worldwide with the aim to evaluate the incidence of food allergy in children across Europe. The adjusted incidence of HEA in the first two years of life was 1.23% on average. Variations were recorded between centers, with UK presenting the highest HEA incidence at over 2% and Greece having the lowest incidence at less than 0.1%. Interestingly, almost half of the perceived HEA reactions were not verified by DBPCFC, further confirming the necessity of objective measurements in the diagnostic algorithm of food allergy. The mean age of children presenting with symptoms of possible and later confirmed HEA was 12 months, with some variation between countries. This might be due to differences in the age at which HE was introduced into the diet, according to national recommendations, although a previous review of weaning recommendations was not indicative of major differences in countries included in the study (14). Furthermore, mean age of children at confirmed diagnosis was 17 months, reflecting the time differences between first presentations of symptoms and confirmed diagnosis.

68.8% of children with suspected HEA eligible for DBPCFC had eczema, highlighting again the strong connection of these two diseases. Almost all children with confirmed HEA showed immediate type symptoms in the food challenge. Finally, HEA seems to have a good prognosis in young children, since almost 50% of our study participants with confirmed HEA tolerated HE within one year.

Comparison with other studies

This is the first study that reported incidence of HEA by the age of two years in a large-scale pan European population. Several epidemiological studies have assessed HEA in different age groups and geographical areas with varying results, depending on data collection

methods and food allergy definitions (15). Although a clear clinical history and sensitization to HE are highly indicative of HEA, the gold standard for diagnosis remains a double-blind, placebo-controlled food challenge test (16-19). Consequently, our results are only directly comparable to studies based on DBPCFC for HEA diagnosis. In such studies, current prevalence (not incidence) estimates of HEA from North-West European regions, ranged from 0.04% in up to 17 year-old German youths (20) to 1.6% in 3 year-old Danish children (21). Results from a prospective UK study using open food challenge tests for diagnosis indicated that the current HEA prevalence at 2 years of age was 0.5% (22). Most comparable to our diagnostic approach was a study from Norway, which used a diagnostic algorithm based on clinical history, SPTs and open or blinded food challenge tests. Eggesbo *et al* estimating a cumulative HEA incidence of approximately 2.6% in early childhood (23). Interestingly, in Australian children, a much higher prevalence of HEA (8.9%) was reported recently (24). The food challenge tests in the Australian children were performed using raw hen's egg, which is comparable in allergenicity to our DBPCFC with pasteurized HE powder. It is known from the Australian population that 80% of the children allergic to raw egg tolerated baked egg, which might be explained by denaturation of the protein (25). Unfortunately, we do not have results on tolerance of baked egg because the DBPCFC tests in EuroPrevall have only been performed with pasteurized raw HE powder but not with baked egg.

Differences in previously reported prevalence estimates were often attributed to differences in study protocols, inclusion criteria or diagnostic techniques. As we have used in our study exactly the same protocols and procedures, we can rule out that these are responsible for observed differences in HEA incidence rates between various European regions. Therefore, other explanations might include genetic factors, different nutritional habits, hygienic, environmental, and socio-economic conditions in the populations.

Immediate type reactions were most frequently observed during the DBPCFC with HE where the majority of children developed urticaria, followed by gastrointestinal and respiratory problems. This was in line with previous reports on organ-specific symptoms during oral food challenge in children with food allergy (26).

Our results indicated a higher remission rate than has been reported in previous, mainly patient-based studies, which showed that HEA children would become tolerant in the course of time (8,9,27). However, our findings are similar to the remission reported at 3

years of age in a population-based study, more comparable to ours, by Venter et al in the UK (22). Differences between our population-based and the patient-based studies' remission rates is likely to be attributable to disease severity differences, as shown by prolonged HEA course in populations referred to tertiary centers (28). Retrospective data analyses have shown that only 4% of HE allergic children become tolerant by the age of 4, a considerably lower percentage compared to our findings; however no regular food challenges were repeated in these populations, possibly overestimating disease duration if based on subjective information (27). For cow's milk allergy, it has been demonstrated that remission estimated from birth cohorts were more optimistic than from allergy clinics (29,30). Our study showed that objective measures are essential in the diagnostic algorithm of food allergies and follow-up, in order to minimize measurement bias and unnecessary avoidance diets (31-33).

Strengths and limitations

Major strengths of our study include its size, the multinational character and its assessment of HE incidence based on a standardized protocol including DBPCFC, the diagnostic gold standard for food allergies. A major limitation of our study was the relatively high proportion of parents who refused their children (44%) being examined by DBPCFC and this might bias results; however, we applied careful adjustments considering possible cases of HEA among eligible children without DBPCF and those lost to follow-up. In respect to development of HE tolerance, it is hypothesized that a proportion of the non-rechallenged HEA children might have consumed HE at home (either accidentally or following their pediatrician's recommendation); however this information was not systematically recorded in the present study and therefore has not been analyzed. Hence, the true proportion/fraction of children becoming tolerant might be even higher.

Conclusion

Our large European cohort study with a comprehensive objective diagnostic work up showed that HEA in early childhood was considerably lower than often perceived by parents or health staff. We estimated the average HEA incidence for European children in the first two years of life to be approximately 1%. Centers in North-Western Europe showed higher incidences (up to 2% in UK) than the centers in Eastern and Southern Europe (lowest in

Greece at 0.1%). HEA had a more favorable prognosis than previously thought with at least half of our children with confirmed HEA became tolerant within one year post-diagnosis. Future research will include follow up of our cohort in school age to evaluate persistence or tolerance development of HEA among the remaining half of affected children as well as new cases of HEA in older children.

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Figure 1. Flowchart of participants included in the egg allergy component within the EuroPrevall birth cohort (DBPCFC = double-blind placebo-controlled food challenge).

*2 of 86 had a convincing history of anaphylaxis and did not actually underwent DBPCFC, but were counted as hen's egg allergy.

Figure 2. Adjusted 2-year incidence of egg allergy by center within the EuroPrevall birth cohort. Total number of children evaluated: 10.218. Bold numbers within countries represent adjusted incidence up to 2 years of age adjusted by center. 95% confidence intervals with adjusted incidence values are presented within the brackets).

Figure 3. Cumulative incidence of hen's egg allergy (solid line with shaded area showing 95% confidence interval at each age) is depicted on the left hand axis with the cumulative percentage of infants introduced to hen's egg in their diet (broken line) on the right hand axis.

Table 1. Demographic and baseline characteristics of study participants. Values are presented as percentage of participants (%), unless otherwise reported. A, asthma; AR, allergic rhinitis; E, eczema

			eligible for DBPCFC		
	all participants (n)	lost to follow up (2y) (n)	not challenged (n)	challenged# (n)	confirmed hen's egg FA (n)
	12049	2713	130	172	86
Demographic characteristics					
Female sex	48.6%	49.1%	46.2%	37.8%	31.4%
Urban living environment	83.9%	81.7%	82.3%	84.3%	76.7%
Smoking in pregnancy	10.6%	14.5%	10.0%	9.3%	8.1%
Age Mother (mean, years)	30.7	29.5	29.3	31.6	32.2
Age Father (mean, years)	33.3	32.6	32.1	33.8	34.0
Highest education of parents					
Low (up to 12y)	16.8%	25.4%	10.8%	8.7%	8.1%
Intermediate (>12y, e.g. college)	33.4%	36.3%	33.1%	33.7%	26.7%
High (e.g. university)	49.3%	38.7%	55.4%	57.6%	65.1%
Allergies in family					
Mother atopic (A, AR or E)	26.2%	26.4%	30.8%	42.4%	43.0%
Father atopic (A, AR or E)	20.7%	18.8%	19.2%	36.6%	39.5%
Mother food allergic	14.1%	13.6%	16.9%	16.9%	18.6%
Father food allergic	8.5%	6.0%	10.8%	12.2%	14.0%
Animals in household					
Any	35.4%	34.4%	37.7%	33.1%	34.9%
Cat	15.1%	14.8%	14.6%	16.3%	19.8%
Dog	16.0%	16.4%	16.2%	8.1%	10.5%
Characteristics at eligibility					
Age of child (mean, months)			13.2	10.6	10.5
Hen's egg specific IgE $\geq$ 0.35 kU/l			46.9%	59.9%	79.1%
Positive SPT ($\geq$ 3 mm, hen's egg)			45.4%	57.0%	64.0%
Eczema			65.4%	70.9%	67.4%

Table 2. Hen's egg allergy follow-up and double-blind placebo-controlled food challenge test (DBPCFC) outcome by center. Values represent numbers of participants' unless otherwise reported. Tolerance development refers to the follow up DBPCFC one year after diagnosis. Raw incidence refers to the first two years of life with the 95% confident interval (CI).

Country	at birth n	hen's egg DBPCFC			tolerance development		age at diagnosis [in months, mean]	challenge lag* [months, in mean]	Confirmed hen's egg allergy	
		Eligible n	Conducted n	reactive (not sensitized) n	Reevaluated n	Tolerant n			raw incidence %	95%-CI %
Iceland	1341	51	42	19	8	2	15	5	1.47	0.89;2.29
United Kingdom	1140	27	20	17	8	2	17	4	1.95	1.14;3.10
The Netherlands	976	33	29	13	10	3	13	2	1.49	0.80;2.54
Germany	1570	33	15	12	6	4	14	5	0.84	0.44;1.47
Poland	1513	40	14	8	5	3	25	7	0.60	0.26;1.17
Lithuania	1556	47	6	2	0	0	19	7	0.15	0.02;0.56
Spain	1387	42	31	9	8	7	16	5	0.78	0.36;1.47
Italy	1486	18	12	5	3	3	15	3	0.44	0.14;1.03
Greece	1080	7	3	1	1	0	15	2	0.11	0.00;0.63
total	1204	298	172	86	49	24	17	4	0.84	0.67;1.03

*interval eligible - challenge.

Table 3: Symptoms in 86 hen's egg reactive double-blind placebo-controlled food challenges at end time-point (either cessation or up to two hours after final dose)

	Symptoms in reactive DBPCFC	n	(%)
Skin			
	Urticaria	47	54.7
	Angioedema	9	10.5
	Flushing	29	33.7
	Eczema (SCORAD * increase <10)	12	14
	Eczema (SCORAD * increase ≥10)	4	4.7
Gastrointestinal			
	Vomiting	30	34.9
	Diarrhoea	4	4.7
	Subjective oral symptoms reported to investigator (pain, nausea, OAS**)	3	3.5
Respiratory and eyes			
	wheeze, dyspnea, cough, or stridor	9	10.5
	Nasal	9	10.5
	Ophthalmic	6	7

* SCORAD = SCORing Atopic Dermatitis (0=best; 100=worst)

** OAS = oral allergy syndrome





