

ORIGINAL ARTICLE

The low prevalence of allergic disease in Eastern Europe

Are Risk Factors Consistent with the Hygiene Hypothesis?

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Experimental
Allergy

Summary

Background The prevalence of allergic disease is known to be low in Eastern Europe.**Objective** To assess the association of suspected risk factors, including several closely linked to the hygiene hypothesis, with allergic symptoms and atopic sensitization in young school-aged children.**Methods** Observational study of 13 889 Belarusian children followed up at age 6.5 years in the Promotion of Breastfeeding Intervention Trial (PROBIT). Allergic symptoms and diseases were based on parental responses to the International Study of Asthma and Allergy in Childhood questionnaire, and prick tests to five common inhalant allergens were performed using standard methods.**Results** Significantly increased risks of wheezing and hayfever symptoms in the past 12 months, and of recurrent itchy rash were observed in boys, children with a positive first-degree family atopic history, and those who had received probiotics (especially as prophylaxis with antibiotic use). Pet ownership, contact with farm animals, the presence and number of younger and (especially) older siblings, and residency in rural areas of Western Belarus were associated with reduced risks. Maternal postnatal smoking was associated with wheezing and hayfever symptoms, while the duration of exclusive breastfeeding was not protective against any of the studied outcomes. The risk factors for allergic symptoms were similar in children with positive skin-prick tests to those in the overall cohort.**Conclusion** Many of the risk and protective factors we identified are consistent with those reported in Western countries and with the hygiene hypothesis. Further research on dietary and other environmental and genetic factors is necessary to understand the low prevalence of allergic disease in Belarus and other Eastern European countries.**Keywords** allergic skin tests, allergy, asthma, atopic eczema, hayfever, hygiene hypothesis*Submitted 5 August 2008; revised 13 November 2008; accepted 16 December 2008*

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Introduction

Despite the increasing prevalence of asthma, hayfever, atopic eczema, and certain food allergies in most Western industrialized countries over the last several decades, the prevalence of allergic disease has remained low in Eastern Europe [1–4]. One of the explanations offered for this East–West difference is the hygiene hypothesis [5, 6]. According to this hypothesis, the lower prevalence in the East is due to frequent infectious disease, lower rates of immunization, and greater exposure to bacterial endotoxins. Moreover, infants and young children in the West are less exposed to microorganisms such as helminths and

saprophytic mycobacteria, which have been part of the normal microflora throughout most of human evolution. This reduced exposure is hypothesized to maintain the T-helper type 2 (Th2) lymphocyte pathway that is predominant at birth from being programmed towards a more adaptive regulatory response [7–11].

Few previous studies have examined the risk factors for the development of allergic disease among children in Eastern Europe [12–15]. It is important to know whether risk factors are similar to or different from those reported in the West, and particularly whether the constellation of risk factors is consistent with the hygiene hypothesis. During the course of designing and conducting the

Promotion of Breastfeeding Intervention Trial (PROBIT), a cluster-randomized trial of a breastfeeding promotion intervention in the Republic of Belarus, we found a very low prevalence of a family history of allergic disease in that country [16]. Yet Belarus and most other former Soviet-bloc countries are characterized by prolonged (usually 3 years) maternity leaves, absence of daycare for children under 3 years of age, high immunization rates, and low fertility rates, and we have previously documented a low incidence of respiratory and (especially) gastrointestinal infection among PROBIT children in the first year of life [16]. Moreover, in keeping with several Western European and North American studies [17–19], we found no protective effect of those infections that did occur against the development of atopic eczema or recurrent wheezing [20].

We recently completed a 6.5-year follow-up of the PROBIT cohort, in which we administered the International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire to the mothers of study children, as well as skin-prick tests (SPTs) for five common inhalant antigens to the children themselves. The results obtained in the primary trial analysis based on intention to treat have been reported recently [21]. Our extensive data on infection during the first year, and additional questions posed at the 6.5-follow-up concerning parental postnatal smoking and past exposure to farm animals, pets, and probiotics, enable us to examine associations expected under the hygiene hypothesis and to gain a better understanding of the risk factors for the development of allergic disease in this setting. To our knowledge, ours is the largest study of allergic disease in childhood ever undertaken in an individual Eastern European country.

Methods

The detailed methods of PROBIT have been reported previously [16, 21], as have the outcomes bearing on allergic disease at the 6.5-year follow-up [21]. In summary, 13 889 (81.5%) of the originally randomized 17 046 children were interviewed and examined at age 6.5 years by paediatricians at the 31 participating polyclinics (out-patient clinics where children are followed for well-child and illness care). Because the rates of prolonged and exclusive breastfeeding were historically higher in West vs. East Belarus and in rural vs. urban areas, treatment allocation was originally randomized within pairs matched by geographic region and rural vs. urban location of the maternity hospital and polyclinic.

As reported previously, data on gastrointestinal and respiratory infection were based on symptom checklists completed at the ages of 1, 3, 6, 9, and 12 months by the polyclinic paediatricians [16]. These symptom checklists were based on occurrences of all symptoms since the previous visit. We adapted the algorithms of Rubin et al.

[22] to convert these symptom checklists into episodes of gastrointestinal and respiratory infection. For the purposes of the current analysis, we analysed the occurrence of one or more episodes of gastrointestinal infection and two or more episodes of respiratory infection in the first 12 months of life in relation to allergic outcomes at 6.5 years. At the time of study enrollment during the post-partum stay at the maternity hospital, the mother was questioned about her own, the father's, and prior siblings' histories of asthma, hayfever, and atopic eczema.

The 6.5-year follow-up took place from December 2002 to April 2005. Allergy symptoms and diagnoses were ascertained with the ISAAC questionnaire, which had already been translated into Russian and validated by the ISAAC investigators [23]. In addition to the ISAAC questionnaire, parents (usually the mother) were questioned about prior exposure to farm animals, ownership of pets, and exposure to probiotics. We also inquired about the reasons the child received probiotics: to relieve abdominal pain ('intestinal colic'), to aid recovery from a diarrhoeal illness, or to prevent diarrhoea at the time of antibiotic treatment. Probiotics in the form of *Lactobacillus* cultures are frequently administered to Belarusian children for these indications.

In addition, the paediatricians performed SPTs to five inhalant antigens: house dust mite, cat, birch pollen, mixed Northern grasses, and *Alternaria* (ALK-Abello, Allergy Canada Ltd., Toronto, ON, Canada). Saline was included as a negative control and histamine (1 mg/mL) as a positive control. The criteria for a positive result were a mean weal ≥ 3 mm or flare ≥ 10 mm, calculated as the mean of the longest and orthogonal diameters after subtracting the mean of these diameters for the saline control [24]. A negative test result required a positive histamine test, defined as a mean diameter with histamine minus the mean diameter with saline ≥ 3 mm for weal or ≥ 5 mm for flare. Of the 13 889 children seen at follow-up, 11 771 (85%) underwent an SPT, of whom 11 145 (95%) had results validated by negative saline and positive histamine responses.

We analysed the crude and adjusted associations between baseline characteristics and other potential risk factors with respect to four allergic outcomes: wheezing in the past 12 months, hayfever symptoms in the past 12 months, recurrent itchy rash, and one or more positive SPTs. The first three of these four outcomes were based on responses to the ISAAC questionnaire. Potentially confounding covariates considered in the analysis included a first-degree atopic family history, maternal age, maternal education, maternal smoking during pregnancy, maternal and paternal smoking at the time of the 6.5-year follow-up (used as a marker for postnatal smoking), geographic region (West vs. East Belarus), urban vs. rural residence, child sex, birth weight, duration of exclusive breastfeeding (< 3 months, 3 to < 6 months, or ≥ 6 months), and

number of older and younger siblings. Multivariate logistic regression was used to analyze each of the dichotomous outcomes based on allergic symptoms and diagnoses from the ISAAC questionnaire and the results of the SPTs using SAS version 9.1 (SAS Institute, Cary, NC, USA), with adjustment for within-polyclinic clustering of measurement for all outcomes.

Results

Table 1 shows the crude associations between the background and exposure variables and the major allergic outcomes under study. Allergic symptoms were infrequent in the study children, but over 20% of them had at least one positive SPT. No statistically significant associations were observed between the allergic outcomes and maternal age, prenatal maternal smoking, paternal smoking, or maternal education. Boys were at a significantly higher risk than girls for most of the allergic outcomes. Postnatal maternal smoking was significantly associated with recent wheezing and recent hayfever symptoms. The prevalence of recent allergic symptoms was higher in Western urban areas of Belarus and lower in Western rural areas, whereas positive SPTs were significantly less frequent in both urban and rural areas of West Belarus.

A higher birth weight was associated with a higher risk of recurrent itchy rash but not with the other outcomes. Pet ownership and contact with farm animals were consistently protective, as were the presence and number of siblings (especially older siblings) in the home. Gastrointestinal and respiratory infections were associated with small increases in risk, especially for respiratory infections and recent wheezing. Exposure to probiotics was associated with statistically significant risk increases for all four of the outcomes under study. The increased risks of allergic symptoms were substantial when the probiotics were taken to treat diarrhoea or to prevent diarrhoea when an antibiotic was prescribed. Family atopic history was significantly associated with all three allergic symptoms, but not with one or more positive SPTs. Consistent with the intention-to-treat results of the intervention trial [21], increased duration of exclusive breastfeeding was not associated with lower risks. In fact, the risks of one or more positive SPTs was higher with exclusive breastfeeding >3 months.

Table 2 shows the results of the multiple logistic regression analyses, which confirmed most of the bivariate relationships seen in Table 1. Gastrointestinal or respiratory infections during infancy were no longer associated with increased risks, but we observed no evidence of a protective effect.

Despite the low prevalence of allergic symptoms among PROBIT children, they were all substantially and significantly more frequent in children with positive SPTs. The prevalence of wheezing in the previous 12 months increased from 2.2% in children with zero positive tests,

6.0% in those with one positive test, and 7.5% in those with two or more positive tests ($P < 0.0001$ based on the χ^2 test for trend). The corresponding prevalences for hayfever symptoms in the previous 12 months were 2.1%, 5.7%, and 9.8%, respectively ($P < 0.0001$), and those for a history of recurrent itchy rash were 3.6%, 7.1%, and 7.0%, respectively ($P < 0.0001$).

Table 3 shows the results of multiple logistic regression analyses for the three allergic symptoms after restriction to those children with one or more positive SPTs. Because of the markedly reduced sample size, the 95% confidence intervals around the adjusted OR estimates are much wider than those shown in Table 2, but the point estimates are strikingly similar, suggesting that the same factors that increase or decrease the risk of allergic symptoms operate both in those with and those without atopic sensitization. The two notable exceptions to this pattern were a positive atopic family history (which was no longer associated with an increased risk of symptoms) and residence in Western urban regions of Belarus, where the increase in risk was even higher among children with positive skin tests.

Discussion

The low prevalence of allergic symptoms and of atopic sensitization (as measured by positive SPTs) in Belarus is consistent with previously published data from other Eastern European countries [1, 3, 6]. We have also confirmed several well-known associations between suspected risk or protective factors and allergic outcomes, including the increased risks associated with male sex, a positive family atopic history, and maternal postnatal smoking (for respiratory allergy) and reduced risks associated with other siblings in the home, rural residence (at least in Western Belarus), pet ownership, and contact with farm animals [25–27]. We also confirmed Matricardi et al.'s [28] observation that the number of older siblings had a larger protective effect (against the ISAAC-based outcomes) than the number of younger siblings.

The absence of any apparent protective effect of infection during infancy confirms our previous report [20] bearing on atopic eczema and recurrent wheezing in the first year of life, and the results of recent studies from the UK and Scandinavia [17–19]. The evidence thus suggests that early exposure to infectious agents, at least those causing common respiratory and gastrointestinal infections in infancy, does not protect against the development of asthma, hayfever, atopic dermatitis, or skin test sensitization.

Other studies, however, suggest that exposure to hepatitis A, helminths, and other pathogens that have historically colonized the gut or the respiratory tract can cause individuals to produce more adaptive immune responses [10, 29, 30]. Regulatory immunity, manifested

Table 1. Crude associations between background and exposure variables and allergic outcomes (in %)

Background/exposure variable (Units)	<i>n</i> (%) in each category	Wheezing in past 12 months	Hayfever symptoms in past 12 months	Recurrent itchy rash	≥ 1 Positive skin-prick test
Maternal age at child's birth (years)					
< 20	1914 (13.8)	3.3	2.8	4.2	20.4
20–34	11 381 (81.9)	3.1	3.3	4.4	22.9
≥ 35	594 (4.3)	2.7	4.4	2.5	21.2
Residence					
West, urban	3353 (24.1)	4.4***	5.0***	6.4***	18.1
West, rural	3779 (27.2)	2.0	1.8	2.2	18.0
East, urban	4500 (32.4)	3.1	3.7	4.0	26.6
East, rural	2257 (16.3)	2.9	2.3	5.0	28.8
Birth weight (g)					
2500–2999	1857 (13.4)	2.8	3.1	3.6*	20.5
3000–3999	10 550 (76.0)	3.1	3.3	4.3	22.9
≥ 4000	1482 (10.7)	3.0	3.4	4.9	22.2
Child sex					
Male	7181 (51.7)	3.5***	3.6*	4.4	22.9
Female	6708 (48.3)	2.6	2.9	4.1	22.1
Atopic family history					
Positive	585 (4.2)	4.6*	6.5***	7.0***	23.6
Negative	13 304 (95.8)	3.0	3.1	4.1	22.5
Prenatal maternal smoking					
Yes	292 (2.1)	3.4	4.8	4.8	17.8
No	13 597 (97.9)	3.1	3.2	4.3	22.6
Maternal smoking at 6.5 years					
Yes	1671 (12.1)	4.2**	4.4**	4.6	23.2
No	12 098 (87.9)	2.9	3.1	4.2	22.5
Paternal smoking at 6.5 years					
Yes	8006 (63.6)	3.0	3.1	4.1	22.3
No	4591 (36.4)	3.1	3.5	4.7	22.9
Pet ownership					
Yes	7218 (52.1)	2.3***	2.3***	3.5***	23.3*
No	6645 (47.9)	3.9	4.3	5.1	21.6
Contact with farm animals					
Yes	8487 (61.2)	2.6***	2.7***	3.6***	20.7***
No	5372 (38.8)	3.8	4.2	5.3	25.4
Probiotic exposure					
For abdominal pain	425 (3.1)	4.2*	6.1***	10.6***	25.8
For diarrhoea	1214 (8.7)	5.3***	6.6***	8.0***	26.7***
With antibiotics	478 (3.4)	10.1***	10.7***	8.8***	27.2***
None	11 775 (85.6)	2.5	2.6	3.5	21.9
Maternal education					
Incomplete secondary	512 (3.7)	2.2	3.3**	4.9	20.5***
Complete secondary	4452 (32.1)	3.3	3.0	4.0	20.7
Advanced secondary or partial university	7088 (51.0)	2.8	3.1	4.2	22.8
Complete university	1837 (13.2)	3.7	4.7	5.1	26.2
Older siblings					
0	7871 (56.7)	3.2*	3.6***	4.8***	23.1*
1	4818 (34.7)	3.2	3.2	3.8	22.1
≥ 2	1200 (8.6)	1.5	1.5	2.3	20.0
Younger siblings					
0	10 460 (75.9)	3.3**	3.6***	4.4	23.1*
1	2969 (21.6)	2.2	2.4	4.0	21.1
≥ 2	348 (2.5)	2.6	2.0	3.2	19.9
Duration of exclusive breastfeeding (months)					
< 3	10 137 (73.0)	3.0	3.2	4.2	21.1***
3–< 6	3228 (23.2)	3.3	3.6	4.3	26.8
≥ 6	524 (3.8)	2.1	2.7	4.4	24.4
≥ 1 GI infection in first year					
Yes	1584 (11.4)	4.1*	3.9	4.3	25.0*
No	12 305 (88.6)	2.9	3.2	4.3	22.2
≥ 2 Respiratory infections in first year					
Yes	5780 (41.6)	3.8***	3.8**	4.6	23.7*
No	8109 (58.4)	2.6	2.9	4.0	21.7

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

Table 2. Results of multiple logistic regression analyses: adjusted ORs (95% CIs)

Background/exposure variable	Wheezing past 12 months	Hayfever symptoms past 12 months	Recurrent itchy rash	≥ 1 Positive skin-prick test
Maternal age at child's birth (years)				
< 20	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
20–34	0.8 (0.6–1.1)	1.4 (1.0–2.0)	1.1 (0.8–1.4)	1.1 (1.0–1.3)
≥ 35	0.9 (0.6–1.5)	1.3 (0.8–2.3)	0.5 (0.3–1.1)	1.1 (0.8–1.4)
Residence				
West, urban	1.6 (1.3–2.1)	1.9 (1.6–2.4)	1.6 (1.3–1.9)	0.6 (0.5–0.7)
West, rural	0.8 (0.6–1.1)	0.7 (0.5–1.0)	0.6 (0.4–0.8)	0.6 (0.5–0.7)
East, urban	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
East, rural	1.6 (1.3–2.2)	1.1 (0.8–1.5)	1.5 (1.2–1.9)	1.2 (1.1–1.4)
Birth weight (g)				
2500–2999	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
3000–3999	1.0 (0.8–1.3)	1.3 (0.9–1.7)	1.3 (1.02–1.8)	1.2 (1.05–1.4)
≥ 4000	1.0 (0.7–1.4)	1.3 (0.9–1.9)	1.6 (1.2–2.3)	1.2 (0.9–1.4)
Male child	1.6 (1.3–2.0)	1.3 (1.1–1.6)	1.1 (0.9–1.2)	1.0 (0.9–1.1)
Positive atopic family history	1.2 (0.9–1.7)	1.7 (1.3–2.2)	1.8 (1.4–2.3)	0.9 (0.7–1.2)
Prenatal maternal smoking	0.9 (0.5–1.6)	1.3 (0.9–2.0)	0.7 (0.4–1.2)	0.8 (0.5–1.2)
Maternal smoking at 6.5 years	1.5 (1.2–1.9)	1.5 (1.2–1.8)	1.2 (0.9–1.5)	1.1 (0.9–1.3)
Paternal smoking at 6.5 years	1.0 (0.8–1.1)	1.0 (0.8–1.1)	1.0 (0.9–1.2)	1.0 (0.9–1.1)
Pet ownership	0.7 (0.5–0.8)	0.7 (0.5–0.8)	0.8 (0.7–0.9)	1.2 (1.1–1.3)
Contact with farm animals	0.7 (0.6–0.8)	0.8 (0.6–0.9)	0.7 (0.6–0.9)	0.8 (0.7–0.9)
Probiotics for abdominal pain	0.9 (0.5–1.4)	1.6 (1.2–2.2)	2.3 (1.8–2.8)	1.1 (0.9–1.5)
Probiotics for diarrhoea	1.8 (1.5–2.2)	2.0 (1.6–2.4)	1.9 (1.6–2.3)	1.2 (1.0–1.4)
Probiotics with antibiotics	3.0 (2.4–3.7)	2.8 (2.3–3.5)	2.2 (1.7–2.8)	1.2 (0.9–1.5)
Maternal education				
Incomplete secondary	0.6 (0.3–1.2)	0.9 (0.5–1.6)	1.6 (1.1–2.3)	0.7 (0.5–1.0)
Complete secondary	1.0 (0.7–1.2)	0.8 (0.6–1.0)	1.0 (0.8–1.3)	0.8 (0.7–0.9)
Advanced secondary or partial university	0.8 (0.6–0.99)	0.8 (0.7–0.98)	1.0 (0.8–1.2)	0.8 (0.7–0.9)
Complete university	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Older siblings				
0	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1	1.2 (1.0–1.5)	1.0 (0.9–1.2)	0.9 (0.8–1.1)	0.9 (0.8–1.0)
≥ 2	0.4 (0.2–1.0)	0.4 (0.2–0.9)	0.7 (0.4–1.1)	0.9 (0.7–1.1)
Younger siblings				
0	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1	0.9 (0.7–1.1)	0.8 (0.6–0.98)	1.2 (1.0–1.4)	0.9 (0.8–1.0)
≥ 2	1.0 (0.5–2.1)	1.1 (0.6–2.1)	0.8 (0.4–1.7)	1.0 (0.7–1.4)
Duration of exclusive breastfeeding (months)				
< 3	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
3 to < 6	1.5 (1.3–1.9)	1.3 (1.1–1.5)	1.1 (0.9–1.3)	1.3 (1.2–1.5)
≥ 6	0.8 (0.4–1.6)	1.3 (0.8–2.0)	1.0 (0.7–1.6)	1.0 (0.8–1.3)
≥ 1 GI infection in first year	1.2 (1.0–1.5)	0.8 (0.6–1.1)	0.8 (0.6–0.99)	1.0 (0.9–1.2)
≥ 2 Respiratory infections in first year	1.1 (0.9–1.3)	1.0 (0.9–1.2)	1.0 (0.9–1.2)	1.0 (0.9–1.2)

OR, odds ratio; CI, confidence interval.

by T-regulatory ($CD4^+CD25^+FOXP3^+$) cells and other cells that produce the regulatory cytokines IL-10 and TGF- β , appears to be crucial for achieving normal immune homeostasis [31–35]. In the extreme, congenital absence of T-regulatory cells is associated with the IPEX syndrome. This frequently fatal mutation has features of both severe autoimmune and severe allergic diseases, suggesting that without T-regulatory cells, both Th1 and Th2 immunity are highly dysregulated [36, 37]. In normal immunity, exposure to low levels of non-infectious mi-

crobes or microbial products such as endotoxins, helminths, and mycobacteria induces the production of IL-10 and TGF- β [38], probably by directly stimulating pattern recognition receptors TLR-2 and TLR-4 on dendritic cells and other cells of the innate immune system [39, 40]. This in turn can reduce the production of IL-4 and other Th2 cytokines [41]. This is a complex system, however, as Th1 and Th2 cells can mutually suppress each other's function; IFN- γ and IL-12 suppress Th2 cytokine production, while IL-4 suppresses Th1 immunity.

Table 3. Results of multiple logistic regression analyses of allergic symptoms among children with ≥ 1 positive skin-prick test; adjusted ORs (95% CIs)

	Wheezing past 12 months	Hayfever symptoms past 12 months	Recurrent itchy rash
Maternal age at child's birth (years)			
< 20	1.0 (reference)	1.0 (reference)	1.0 (reference)
20–34	0.8 (0.4–1.4)	1.3 (0.8–2.3)	0.8 (0.5–1.4)
≥ 35	1.2 (0.5–3.2)	2.2 (0.9–5.6)	0.4 (0.1–1.3)
Residence			
West, urban	2.1 (1.4–3.1)	1.8 (1.3–2.7)	2.0 (1.3–3.0)
West, rural	0.8 (0.5–1.4)	0.6 (0.3–0.96)	0.7 (0.4–1.2)
East, urban	1.0 (reference)	1.0 (reference)	1.0 (reference)
East, rural	0.8 (0.5–1.5)	0.5 (0.3–0.9)	1.0 (0.6–1.7)
Birth weight (g)			
2500–2999	1.0 (reference)	1.0 (reference)	1.0 (reference)
3000–3999	0.9 (0.5–1.6)	1.2 (0.7–1.9)	1.4 (0.8–2.4)
≥ 4000	1.0 (0.5–2.0)	1.1 (0.5–2.2)	1.8 (0.8–3.7)
Male child	1.2 (0.9–1.7)	1.2 (0.9–1.7)	0.8 (0.6–1.2)
Positive atopic family history	0.9 (0.4–2.1)	0.7 (0.3–1.7)	1.3 (0.6–2.8)
Prenatal maternal smoking	0.3 (0.0–2.5)	0.5 (0.1–2.2)	0.9 (0.2–3.1)
Maternal smoking at 6.5 years	1.5 (0.9–2.5)	1.5 (1.0–2.4)	1.2 (0.7–2.0)
Paternal smoking at 6.5 years	0.9 (0.6–1.3)	1.1 (0.8–1.5)	1.1 (0.7–1.5)
Pet ownership	0.6 (0.4–0.8)	0.6 (0.4–0.8)	0.8 (0.6–1.2)
Contact with farm animals	0.8 (0.6–1.1)	0.7 (0.5–1.0)	0.7 (0.5–0.96)
Probiotics for abdominal pain	1.5 (0.7–3.3)	2.4 (1.2–4.6)	2.2 (1.1–4.4)
Probiotics for diarrhoea	1.7 (1.04–2.7)	2.2 (1.4–3.5)	1.8 (1.1–3.0)
Probiotics with antibiotics	2.9 (1.6–5.2)	2.8 (1.6–4.9)	2.0 (1.03–3.9)
Maternal education			
Incomplete secondary	0.3 (0.1–1.5)	1.0 (0.4–2.8)	0.8 (0.3–2.3)
Complete secondary	0.7 (0.4–1.2)	0.8 (0.5–1.4)	0.7 (0.4–1.3)
Advanced secondary or partial university	0.8 (0.5–1.2)	0.8 (0.6–1.3)	0.8 (0.5–1.2)
Complete university	1.0 (reference)	1.0 (reference)	1.0 (reference)
Older siblings			
0	1.0 (reference)	1.0 (reference)	1.0 (reference)
1	1.1 (0.8–1.7)	1.0 (0.7–1.4)	0.8 (0.6–1.2)
≥ 2	0.6 (0.2–1.5)	0.5 (0.2–1.2)	0.8 (0.3–1.8)
Younger siblings			
0	1.0 (reference)	1.0 (reference)	1.0 (reference)
1	0.7 (0.4–1.1)	0.8 (0.5–1.2)	1.1 (0.7–1.7)
≥ 2	1.4 (0.4–4.9)	0.8 (0.2–3.3)	1.2 (0.4–4.2)
Duration of exclusive breastfeeding (months)			
< 3	1.0 (reference)	1.0 (reference)	1.0 (reference)
3 to < 6	1.5 (1.0–2.1)	0.8 (0.5–1.1)	0.7 (0.4–1.0)
≥ 6	0.8 (0.3–2.3)	1.1 (0.5–2.4)	0.6 (0.2–1.8)
≥ 1 GI infection in first year	1.2 (0.8–2.0)	0.7 (0.4–1.2)	0.9 (0.6–1.6)
≥ 2 Respiratory infections in first year	1.1 (0.8–1.6)	1.1 (0.8–1.5)	1.3 (0.9–1.8)

OR, odds ratio; CI, confidence interval.

Importantly, the incidences of both Th2 (allergic) and Th1 (autoimmune) diseases have increased in parallel with the decline in many infectious diseases that appear to contribute strongly to immune regulation [9].

Most Belarusian children (and those in other former Soviet-bloc countries) remain at home with their mothers for the first 3 years of life. The standard maternity leave in Belarus is 3 years, and day care is unavailable for children under 3 years. Along with low fertility rates, small family sizes, and high breastfeeding rates, these conditions probably contribute to the low incidence of infectious illness

during infancy that we previously documented in the PROBIT cohort [16]. The low infection incidence obviously cannot explain the low incidence of allergic disease. On the other hand, the protective effects we observed for the presence and number of older siblings, pet ownership, and contact with farm animals are consistent with previous epidemiologic studies from North America and Western Europe and with the hypothesis that exposure to bacterial infection, animal feces, or other source of endotoxin may confer protection against the risk of allergic disease. Unfortunately, no information is

available on the specific latter exposures in PROBIT children, and the absence of data on the chronologic sequence of events prevents us from knowing whether the inverse associations between allergic disease and pet ownership or contact with farm animals might reflect reverse causality, i.e., the tendency of parents of children with allergic disease to avoid these exposures.

We have no obvious explanation for the higher risk of allergic symptoms in Western urban regions of Belarus, nor the reduced risk in Western rural areas. Each of the four regions contains five to 10 polyclinics participating in PROBIT, with little or no regular contact among the paediatricians or study subjects. Western regions tend to resemble Poland and the Baltic countries in diet, behaviour, and other life-style factors, whereas Eastern regions are closer to Russia and Ukraine in these respects. The fact that these regional differences persisted even after adjustment for pet ownership, contact with farm animals, and probiotic use suggests that unmeasured genetic and/or environmental factors may be responsible. Such factors clearly merit further study.

The higher prevalence of SPT positivity than of recent allergic symptoms suggests that atopic sensitization may precede the development of allergic symptoms. Skin test positivity has been reported to precede the onset of allergies in US college students by as long as 10–20 years [42, 43]. Moreover, studies in other Eastern European countries have also reported high rates of skin-prick positivity but low rates of asthma, hayfever, and eczema [44–46]. In this context, it will be interesting to see whether allergic symptoms and diagnoses increase in Belarus and other Eastern European countries in future and whether their prevalence begins to approach that in the West, as has been reported in Estonia [47].

As shown in Table 3, most of the risk factors we studied had similar associations with allergic symptoms among the 22% of children with one or more positive SPTs as in the entire study sample. Because associations with symptoms were stronger than for positive skin tests (Table 2), these results suggest that the studied factors affect the risk of symptoms irrespective of atopic sensitization. This conclusion should be tempered, however, by the recognition that SPT reactivity and symptoms were ascertained at the same point in time (the study visit at age 6.5 years) and thus may not predict the development of future symptoms among children with positive tests.

We were surprised to observe that past exposure to probiotics was associated with increased, rather than reduced, risks of allergic symptoms. In Belarus, probiotic agents (usually in the form of *Lactobacillus* cultures) are given to relieve abdominal pain ('intestinal colic'), to speed recovery from diarrhoeal diseases, and to prevent diarrhoea at the time of antibiotic treatment. Unfortunately, we have no information on the timing of probiotic administration vis-à-vis the first development of allergic

symptoms. The fact that we observed the highest risks when probiotics were given prophylactically at the onset of antibiotic treatment suggests that the antibiotics, or the bacterial or other infections that led to the antibiotic treatment, may be the true causal factor [48]. It is also possible that probiotic use is merely a marker for life-style or behavioural characteristics that increase the risk of allergic disease, rather than an independent cause. Antibiotics are prescribed frequently in Belarus to treat non-specific respiratory infections, thus increasing exposure to both antibiotics and probiotics. A recent Cochrane review found no strong evidence that prophylactic treatment with probiotics in infancy reduces the risk of allergic disease [49]. Moreover, a recent trial of maternal prophylaxis beginning in late pregnancy actually observed an increased risk among offspring of probiotic-treated mothers [50].

In summary, despite a large sample size, prospective follow-up, and detailed information on the potential risk factors and allergic outcomes, we have no obvious explanation for the low prevalence of allergic disease in Belarusian children. Genetic differences seem unlikely, given the large difference in prevalence previously documented between East and West Germany [6], but should be explored. Variations within and between populations, and the temporal increase in allergic disease in the West, may be linked to understudied factors such as dietary differences. The typical Eastern European diet is rich in animal fat, and fish is too expensive for many, resulting in relatively high intakes of n-6 long-chain polyunsaturated fatty acids and correspondingly low intakes of n-3 fatty acids [51]. Some evidence suggests that such dietary differences can affect the risk of wheeze and asthma [52]. Nutritional and other differences between Eastern and Western European populations clearly merit further study.

Acknowledgements

Drs Michael S. Kramer, Lidia Matush, Natalia Bogdanovich, Robert W. Platt, and Bruce Mazer designed the study, planned the field work and data collection, and participated in the drafting and/or revision of the manuscript. Drs Kramer and Platt and Mr Mourad Dahhou carried out the statistical analysis.

Supported by a grant from the Canadian Institutes of Health Research. Dr Platt is a career investigator (chercheur-boursier), and Dr Mazer a chercheur national, of the Fonds de la recherche en santé du Québec (FRSQ). Drs Kramer, Platt, and Mazer and Mr Dahhou are members of the Research Institute of the McGill University Health Centre, which is supported in part by the FRSQ.

None of the authors has any conflict of interest of any nature concerning the topic or contents of this article.

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