

Original article

Specific IgE to allergens in cord blood is associated with maternal immunity to *Toxoplasma gondii* and rubella virus

Background: Various studies have found reduced prevalences of atopic sensitization and atopic diseases in children previously exposed to infections or living conditions with a high microbial burden, such as the farming environment.

Objective: We sought to determine the relationships of cord blood immunoglobulin E (IgE) with maternal health conditions before and during pregnancy.

Methods: Pregnant women living in rural areas in five European countries were recruited in the third trimester of pregnancy. Information on maternal health during pregnancy was collected from maternity records and by questionnaires ($n = 497$). Specific IgE for inhalant and food allergens was assessed in cord blood and peripheral blood samples of the mothers.

Results: Inverse associations of cord blood IgE to seasonal allergens with positive maternal records for *Toxoplasma gondii* (adjusted odds ratio = 0.37 [0.17–0.81]) and *rubella virus* (adjusted odds ratio = 0.35 [0.13–0.96]) were found. The previously described effect of prenatal farm exposure on IgE to seasonal allergens was partly confounded by a positive maternal record for *T. gondii*. The number of maternal siblings, maternal contact to cats during pregnancy or during her first year of life, predicted a positive maternal record for *T. gondii*.

Conclusions: Maternal immunity to *T. gondii* and rubella may impact on atopic sensitization in the fetus. A positive *T. gondii* record explained the previously identified effect of prenatal farm exposure on IgE to seasonal allergens only to a minor extent.

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In 1976, Gerrard et al. proposed that 'relative freedom from infectious diseases in childhood ... might lead to an increase in the prevalence of atopic disease' (1), a notion which later emerged as the 'hygiene hypothesis' (2). In the meantime, many surveys and aggregate studies on infections and atopy have generated data compatible with this hypothesis: some referred to specific infections during childhood such as infection with *Toxoplasma gondii* (3–6), whereas others brought up the idea of a continuous exposure during childhood to environments rich in microbial burden such as the farming environment (3, 7–11). One of these publications proposed infection with *T. gondii* as an intermediate step in the association of farming exposure in childhood with decreased rates of atopic diseases (3). This assumption, however, was based on a retrospective analysis of adults; additional data in children would be desirable, as exposure to farming has been observed to operate early in life (11). The PASTURE birth cohort (12) including children from rural areas in five European countries now provides the opportunity to study both farming environment and maternal immune status to particular infectious agents such as *T. gondii* and rubella virus. A previous analysis of this cohort revealed differential sensitization patterns for farm and reference children, as determined by immunoglobulin (Ig) E measurements in cord blood (CB) (Ege MJ, Herzum I, Büchele G, Krauss-Etschmann S, Lauener RP, Roponen M, Hyvärinen A, Vuitton DA, Riedler J, Brunekreef B, Dalphin JC, Braun-Fahrlander C, Pekkanen J, Renz H, von Mutius E, the PASTURE study group, unpublished data). The relevant farm exposures associated with lower prevalence of IgE against seasonal allergens in that analysis were 'exposure to animal sheds during pregnancy' and 'the presence of an open dung hill in the surrounding area'.

The objective of the present analysis was to elucidate the relation of specific CB IgE to the maternal health status with a focus on infections before and during pregnancy.

Methods

Study design and population

The PASTURE study was carried out as described previously (12). Pregnant women living in rural areas in Austria, Finland, France, Germany, and Switzerland were recruited in the third trimester of pregnancy. Women who lived on farms where any kind of livestock was kept were assigned to the farm group. For the reference group, women from the same rural areas, but not living on a farm were recruited.

Maternity records

Data on documented measurements of antibodies to *T. gondii*, rubella virus, and hepatitis B virus surface antigen (HBsAg) were collected from maternity records as yes/no answers. Discrete titers were not available. Tests and cut-off values applied varied across and within study regions. At the Max-von-Pettenkofer-Institute

in Munich (<http://www.mvp.uni-muenchen.de/fileadmin/mvp/pdf/mikro-diag.pdf>), e.g., *T. gondii* screening is performed by ELISA tests for IgG and IgM antibodies. In case of positive or borderline results, immunofluorescence tests are performed. For rubella screening, usually a hemagglutination inhibition test is applied and in case of lower titers, it is followed by an ELISA test for rubella IgG. *Toxoplasma gondii* and rubella virus are not routinely screened in Finland, and no measurements were carried out in the Finnish study sample; therefore, Finnish children were excluded from the present analysis.

Questionnaires

The questionnaires used within the PASTURE study group were based on the International Study of Allergy and Asthma in Childhood (ISAAC) (13), the ALEX (Allergy and Endotoxin study) (10), and the PARSIFAL study (Prevention of Allergy Risk factors for Sensitization In children related to Farming and Anthroposophic Lifestyle) (14). Questionnaires were administered to the mothers at the end of pregnancy and when the children were 2 months of age. The questions referred to the general health of the children's families with a focus on respiratory and atopic diseases and maternal health during pregnancy. Furthermore, questions were asked about the intensity and timing of maternal farm-related exposures and maternal nutrition during pregnancy, e.g., consumption of raw milk or meat and sausages from own production (at least once a week). Potential confounders were addressed as well. Both parents were also asked for the number of their siblings, whether they grew up on farms, and whether they were exposed to pets during their first year of life.

Specific IgE in serum samples

Specific IgE for seven food and 13 common inhalant allergens was assessed in CB at birth by the Allergy Screen test panel for atopy (Mediawiss Analytic, Moers, Germany) as described previously (15). In addition, peripheral blood samples of the mothers taken at birth or during a home visit, when the child was 2 months old, were analyzed.

Statistical analysis

Statistical analysis was performed with SAS 9.1.3 (The SAS Institute, Cary, NC, USA). Crude (cOR) and adjusted (aOR) odds ratios are given with 95% confidence intervals. Specific CB and maternal peripheral blood IgE levels were dichotomized at the detection limit of 0.2 IU/ml. Combinations of specific IgE were defined: IgE to food allergens (hen's egg, cow's milk, peanut, hazelnut, carrot, wheat flour, soybean); IgE to perennial allergens (*Dermatophagoides pteronyssius*, *Dermatophagoides farinae*, cat, horse, dog); and IgE to seasonal allergens (alder pollen, birch pollen, hazel pollen, rye pollen, grass pollen mix, Mugwort, plantain, *Alternaria*).

Maternal health-related characteristics were compared between farm and reference mothers by Fisher's exact test. Associations of positive maternal *T. gondii*, rubella, and HBsAg records with CB IgE to inhalant (seasonal or perennial) and food allergens were explored in logistic regression adjusted for study center and farming. Models for competing effects of positive *T. gondii* and positive rubella records on IgE to seasonal or perennial allergens were adjusted for potential confounders such as, maternal IgE to seasonal or perennial allergens, respectively, maternal smoking during pregnancy, mode of birth, number of previous pregnancies, contact to pets during pregnancy, gender, farming, center, maternal and paternal history of atopic diseases, educational level, and

farm exposure during their childhood. Models for IgE to seasonal allergens were additionally adjusted for previously identified farm-related determinants. A parsimonious model for IgE to seasonal allergens was established by stepwise logistic regression ($P < 0.15$) to study the mutually adjusted effects of farm-related exposures and the positive *T. gondii* and rubella records without adjustment for farming. Potential maternal determinants of a positive *T. gondii* record were explored in stepwise logistic regression ($P < 0.15$), and the retrieved variables were tested for modifying the association of *T. gondii* and CB IgE. In a sub-sample analysis, the effects of *T. gondii* and rubella on IgE to seasonal allergens were assessed separately for children, whose mothers were or were not exposed to cats in their own first year of life or during pregnancy.

Results

Of the 2871 women contacted, 1772 (62%) were eligible for the PASTURE study, and of these, 64% were willing to participate resulting in 1133 recruited newborns (Fig. 1). In 922 mother/child pairs maternal and CB IgE values were available. The 497 mother/child pairs with complete records for *T. gondii* and rubella records are subject to this analysis and will be referred to as 'analysis sample'. The analysis sample did not differ from the 922 mother/child pairs with IgE values with respect to farming (Fig. 1) or parental history of atopic diseases. Austrian and French participants were more and German participants were less prevalent in the analysis sample; Finnish participants were absent in the analysis sample (Fig. 1). The following characteristics were more prevalent in the analysis sample: CB IgE to seasonal

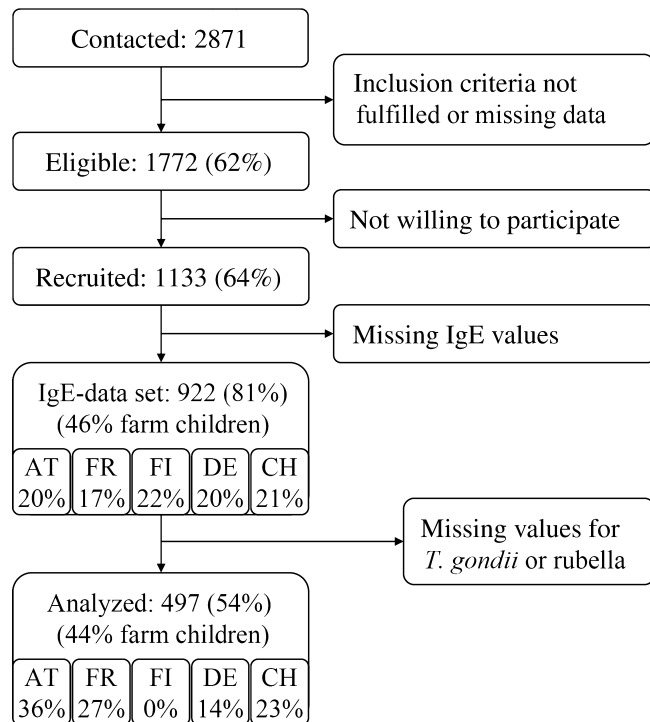


Figure 1. Recruitment of the study population.

allergens, higher paternal education, primigravids, pregnancy edema, gestational diabetes, and positive rubella records. All these differences were explained by the 'center' variable (data not shown); therefore, subsequent models were adjusted for center.

The distribution of health characteristics between farm and reference mothers is given in Table 1. Pregnancy-related complications such as edema, diabetes or hypertension; infections; and the use of antibiotics did not vary between these groups. A positive *T. gondii* record, however, was more prevalent in farm mothers (cOR = 1.74 [1.20–2.50]). The country-specific *T. gondii* prevalence was 23% for Germany, 31% for France, 41% for Switzerland, and 48% for Austria.

Prevalence of atopic diseases and atopic sensitization was higher in reference mothers (Table 1). As previously reported, IgE to seasonal allergens in CB was less prevalent in farm children (5% vs 11%, $P < 0.001$), and IgE to food allergens was more prevalent in farm children (19% vs 14%, $P = 0.032$). For IgE to perennial allergens, farm and reference children did not differ.

As CB IgE and positive maternal *T. gondii* records varied between the farm and reference group, associations of CB IgE with maternal records were explored (Table 2). Inverse associations were found for *T. gondii* and rubella with IgE to inhalant allergens, in particular seasonal allergens. For IgE to food allergens, no association was found. Crude prevalences are shown in Table 3.

In multivariable analysis, the inverse relations of positive *T. gondii* and rubella records with IgE to seasonal allergens persisted (Table 4). The variable 'farm vs reference group' did not exert an inverse effect on CB IgE to seasonal allergens, when adjusted for positive *T. gondii* and rubella records and the two previously identified farm-related determinants 'exposure to animal

Table 1. Health characteristics of farm ($n = 219$) and reference mothers ($n = 278$)

	Farm mothers, <i>n</i> (%)	Reference mothers, <i>n</i> (%)	<i>P</i> -value
Primigravida	57 (26)	121 (44)	<0.001**
Preterm birth	1 (0)	5 (2)	0.233
Spontaneous birth	186 (85)	209 (77)	0.023*
Pregnancy edema	77 (35)	99 (36)	0.850
Gestational hypertension	15 (7)	23 (8)	0.611
Gestational diabetes	14 (6)	25 (9)	0.315
Airway infection in pregnancy	151 (68)	171 (62)	0.157
Vaginal infection in pregnancy	41 (19)	56 (20)	0.650
Use of antibiotics in pregnancy	44 (20)	54 (20)	1.000
Positive <i>Toxoplasma gondii</i> record	100 (45)	89 (32)	0.004**
Positive rubella record	197 (89)	253 (92)	0.358
Positive HBsAg record	4 (2)	6 (3)	0.760
Maternal history of asthma	16 (7)	25 (9)	0.514
Maternal history of atopic diseases	45 (20)	93 (34)	0.001**
Maternal IgE to any allergen	142 (64)	201 (73)	0.041*
Maternal IgE to seasonal allergens	75 (34)	119 (43)	0.042*
Maternal IgE to perennial allergens	89 (40)	131 (47)	0.123
Maternal IgE to food allergens	87 (39)	97 (35)	0.351

* $P < 0.05$; ** $P < 0.001$.

Table 2. Associations of cord blood IgE with maternal records ($n = 497$)

Positive record	IgE to inhalant allergens	IgE to seasonal allergens	IgE to perennial allergens	IgE to food allergens
<i>Toxoplasma gondii</i>	0.46 [0.25–0.84], $P = 0.011$	0.40 [0.20–0.79], $P = 0.009$	0.77 [0.28–2.13], $P = 0.618$	0.80 [0.46–1.40], $P = 0.439$
Rubella	0.45 [0.19–1.06], $P = 0.068$	0.39 [0.16–0.96], $P = 0.040$	0.64 [0.13–3.21], $P = 0.585$	1.35 [0.51–3.56], $P = 0.541$
HBsAg ($n = 441$)	2.42 [0.58–10.2], $P = 0.227$	1.96 [0.38–10.1], $P = 0.422$	5.08 [0.87–29.8], $P = 0.072$	2.45 [0.52–11.6], $P = 0.257$

The OR are adjusted for farming and center, but not mutually. OR are given with 95% confidence intervals in brackets.

Table 3. Prevalence (%) of cord blood IgE for children of mothers with positive/negative records for *Toxoplasma gondii* and rubella ($n = 497$)

	<i>T. gondii</i>		Rubella	
	Positive ($n = 189$)	Negative ($n = 308$)	Positive ($n = 450$)	Negative ($n = 47$)
IgE to inhalant allergens ($n = 74$)	9.0	18.5	14.4	19.1
IgE to food allergens ($n = 80$)	15.3	16.6	16.4	12.8

sheds during pregnancy' and 'presence of an open dung hill in the surrounding area' (Model M1 in Table 4). Also in a parsimonious model without the variable 'farm vs reference group', the positive *T. gondii* and rubella records did not explain the two farm-related determinants (Model M2 in Table 4), but adjustment for a positive *T. gondii* or rubella record changed the estimates for 'exposure to animal sheds during pregnancy' by about 18% (Models M2 and M3, Table 4). The variable 'open dung hill in surrounding area' stayed in the model with borderline statistical significance, and no relevant change-in-estimate was noted (Table 4).

In a subsequent step, determinants for a positive *T. gondii* record in the mother were explored. The number of maternal siblings, maternal contact to cats during pregnancy and during the mother's first year of life were identified as mutually independent determinants of a positive *T. gondii* record (Table 5). An association of farming *per se* with a positive *T. gondii* record disappeared after adjustment for these other determinants. The consumption of meat and sausages from own production could not clearly be ruled out as a determinant for a positive *T. gondii* record; yet, other variables such as consumption of raw farm milk were not related to a positive *T. gondii* record (data not shown).

Table 4. Mutually adjusted models for cord blood IgE to seasonal allergens and maternal records ($n = 458$)

Exposure	M1 ($n = 458$)	M2 ($n = 475$)	M3 ($n = 475$)
Positive toxoplasma record	0.33 [0.14–0.80], $P = 0.014$	0.37 [0.17–0.81], $P = 0.013$	
Positive rubella record	0.34 [0.11–0.98], $P = 0.046$	0.35 [0.13–0.96], $P = 0.041$	
Farm vs reference group	1.29 [0.41–4.08], $P = 0.665$		
Exposure to animal sheds during pregnancy	0.46 [0.19–1.15], $P = 0.098$	0.46 [0.22–0.96], $P = 0.038$	0.39 [0.19–0.77], $P = 0.007$
Open dung hill in surrounding area	0.53 [0.24–1.16], $P = 0.111$	0.56 [0.29–1.10], $P = 0.092$	0.59 [0.31–1.13], $P = 0.111$
Maternal IgE to respective allergens	1.05 [0.54–2.05], $P = 0.878$		

All models are adjusted for the other exposures listed in the respective columns of Table 4 and additionally for center and season of birth. Model M1 is additionally adjusted for gender, mode of birth, number of previous pregnancies, maternal smoking during pregnancy, contact to pets during pregnancy, maternal and paternal history of atopic diseases, maternal and paternal educational level, maternal and paternal farm exposure during childhood. OR are given with 95% confidence intervals in brackets.

Table 5. Determinants of positive maternal *Toxoplasma gondii* records ($n = 479$)

Determinant	Positive <i>T. gondii</i> records
Maternal contact to cats during her own first year of life	2.22 [1.45–3.39], $P < 0.001$
Maternal contact to cats during pregnancy	1.77 [1.12–2.81], $P = 0.015$
Number of maternal siblings	1.20 [1.06–1.36], $P = 0.003$
Consuming meat and sausages from own production	1.49 [0.93–2.39], $P = 0.100$
Farm vs reference group	0.94 [0.57–1.56], $P = 0.819$

The OR are adjusted for the other determinants listed in Table 5 and additionally for center. OR are given with 95% confidence intervals in brackets.

The association of a positive *T. gondii* record with IgE to seasonal allergens in the offspring, as presented in Table 4 (M2), withstood adjustment for these maternal determinants of a positive *T. gondii* record (aOR = 0.42 [0.19–0.94]), thereby supporting a proper effect of a positive maternal *T. gondii* record on CB IgE.

As maternal infancy exposure to cats was the strongest predictor for a positive *T. gondii* record (Table 5), it was hypothesized that in most *T. gondii*-positive mothers, the infection might have occurred early in their lives. When analyzing the effect of a positive *T. gondii* record on IgE to seasonal allergens in strata with or without maternal infancy exposure to cats, the aOR became stronger in the stratum *with* maternal infancy exposure to cats and was closer to unity in the stratum *without* maternal infancy cat exposure (Table 6). The same pattern was seen when stratifying for number of maternal siblings (data not shown). In mothers who had contact to cats during pregnancy but not in infancy ($n = 78$), there was no inverse association for a positive *T. gondii* record with CB IgE to seasonal allergens (1.81 [0.32–10.3], $P = 0.505$).

Table 6. Effect of positive maternal *Toxoplasma gondii* records on IgE to seasonal allergens in the offspring stratified for contact to cats in the mothers' first year of life

Exposure	With childhood cat contact	Without childhood cat contact
Positive <i>T. gondii</i> record	0.12 [0.03–0.57], <i>P</i> = 0.007	0.86 [0.33–2.23], <i>P</i> = 0.761
Positive rubella record	0.95 [0.23–3.94], <i>P</i> = 0.942	0.31 [0.10–0.95], <i>P</i> = 0.040
Prenatal exposure to animal sheds	0.29 [0.09–0.91], <i>P</i> = 0.034	0.48 [0.21–1.08], <i>P</i> = 0.077

Adjusted OR are given for sub-samples with (*n* = 215) and without (*n* = 248) contact to cats in maternal childhood. The models are mutually adjusted and additionally for center and maternal IgE to seasonal allergens. OR are given with 95% confidence intervals in brackets.

A sensitivity analysis using logistic regression weighted and stratified for the study centers led basically to the same results as the center-adjusted analysis. The weighted stratified OR for rubella infection were weaker and not significant in Table 2, but remained unchanged in the other tables. The effect of an open dunghill was stronger and significant in stratified weighted analysis (data not shown).

Discussion

The present analysis of the PASTURE birth cohort revealed inverse associations of positive maternal records to *T. gondii* and rubella virus with CB IgE to seasonal allergens, but not to perennial inhalant or food allergens. The associations persisted in multivariable models. A positive *T. gondii* record explained only a small part of the previously identified effect of 'exposure to animal sheds during pregnancy' on IgE to seasonal allergens. As determinants for a positive maternal record for *T. gondii*, the number of maternal siblings, maternal contact to cats during her first year of life and during pregnancy, and possibly consumption of meat from own production, were identified. The effect of a positive maternal record for *T. gondii* on CB IgE to seasonal allergens was enhanced by maternal contact to cats during her first year of life.

The feasibility and validity of IgE measurement at a low detection level in CB have been shown previously (15). Contamination of CB by maternal blood occurred only in 5% of children (Pfefferle P, Sel S, Ege MJ, Büchele G, Bluemer N, Krauss-Etschmann S, Herzum I, Albers CE, Lauener RP, Roponen M, Hirvonen MR, Vuitton DA, Riedler J, Brunekreef B, Dalphin JC, Braun-Fahrlander C, Pekkanen J, van Mutius E, Renz H, the PASTURE study group, unpublished data) and was controlled in the present analyses by adjustment for maternal IgE to the respective allergens. The validity of exposure assessment may be disputed, because *T. gondii* and rubella antibodies were not analyzed centrally, but collected from maternity records. However, these mea-

surements are routine screening procedures, are well documented, and their usefulness has been proven in many years of practical application. Cut-off definitions may vary across countries and laboratories; yet, this variation is random and may only lead to decreased precision and underestimation of the effect. In any case, potential misclassification of exposure is independent of the study endpoints. With respect to complete values for *T. gondii* and rubella records, selection by center may have occurred. However, a weighted sensitivity analysis stratified by center led to the same conclusions.

In the context of the 'hygiene hypothesis', an association of a previous measles infection with a reduced prevalence of atopic sensitization has been discussed controversially (5, 16–18). The present analysis is the first to find a positive rubella record to be associated with reduced atopic sensitization. Yet, data on other viral infections such as measles or mumps were not available. As vaccination against rubella has been propagated in Europe since the late 1960s, detection of rubella antibodies may not only reflect natural immunity, but also previous vaccination. Moreover, combined vaccines against measles, mumps, and rubella have been used increasingly. Therefore, a specific effect of established rubella immunity cannot be disentangled from vaccination against measles or mumps in our study population.

In contrast, vaccination against *T. gondii* does not exist; therefore, detection of specific antibodies reflects a previous infection. *Toxoplasma gondii* infections of humans are mostly asymptomatic, but may have deleterious effects in immunocompromised patients or when acquired congenitally (19). The latter condition arises from maternal *de novo* infection during pregnancy; therefore, screening before or in early pregnancy is recommended. Because of their widespread shedding of infectious oocysts of *T. gondii*, domestic cats are regarded as the major source of infection in humans (19). A further relevant route of transmission is consumption of raw or undercooked meat (19).

The different prevalence between farm and reference mothers (Table 1) suggested an association of a *T. gondii* infection with farming. A multivariate analysis, however, revealed confounding of the suspected association by number of maternal siblings and maternal contact to cats during pregnancy or infancy (Table 5). After all, a previous *T. gondii* infection changed the estimate of the effect of farm exposure during pregnancy on IgE to seasonal allergens by more than 10%, but was far from explaining the effect completely. The data set did not provide information on consumption of undercooked meat, but the variable 'consuming meat and sausages from own production' may comprise consumption of fumed meat and sausages, which usually are not cooked. This may explain the nonsignificant association with a positive *T. gondii* record.

The question remains why only IgE to seasonal allergens in CB is affected by positive records for *T. gondii*

and rubella. In any case, the finding is paralleled by the phenomenon that pregnancy exposure to animal sheds exerted its protective effect only on CB IgE to seasonal allergens in the same study population. Potential explanations might be found in the structure of the allergens, their antigenicity, quantitative occurrence, or context of exposure. In the rural study population, the ubiquitous sources of grass pollen (and their cross-reactivity with tree pollen) may play a major role in sensitization to seasonal allergens compared with perennial or food allergens.

Furthermore, detection of specific CB IgE at low levels does not necessarily predict manifestation of atopic disease. The presence of specific IgE, however, may be a sign of a previous immune reaction. A link between a specific IgE response and immunity towards *T. gondii* may be found in toll-like receptors. These receptors of the innate immunity recognize *T. gondii* antigens (20) and are capable to shift the balance from T-helper 2 to T-helper 1 cells (21).

In this study, previous infection with *T. gondii* exerted its effect only in the stratum of mothers who were already exposed to cats during their first year of life, but not in mothers exposed only recently. This observation is paralleled by the effect modification by number of maternal siblings. Despite low case numbers in the strata, these findings may be of interest as exposure to cats in

early life or exposure to siblings may be a proxy for a *T. gondii* infection in early life. The retrospective assessment of maternal contact to cats in early infancy may have resulted in recall bias. Yet, the blinded outcome of CB IgE and the direction of the effect, i.e. an *inverse* association, argue against differential recall. In face of cautious interpretation, the association of a maternal *T. gondii* infection in infancy with reduced IgE production against seasonal allergens in the offspring might reflect an epigenetic effect.

Taken together, the present analysis of the PASTURE birth cohort revealed an inverse association of CB sensitization to seasonal allergens with established maternal immunity against rubella and *T. gondii*. A positive record for *T. gondii* partly explained the inverse association of farming with atopic sensitization to seasonal allergens. Further elucidation of the protective farming effect on atopy may help to find a way to 'relative freedom from infectious diseases in childhood and atopic disease.'

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