

Farming exposure in childhood, exposure to markers of infections and the development of atopy in rural subjects

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Abstract

Background Within the context of the hygiene hypothesis, we aimed to study the potential association between farming-related risk factors and *Toxoplasma gondii* (*T. gondii*) as well as *Helicobacter pylori* (*H. pylori*) seropositivity.

Methods The study included questionnaire data and serum samples of 321 young adults living in a rural environment. Serum samples were analysed for specific IgE to a common panel of aeroallergens (SX1) as well as IgG against *T. gondii* and *H. pylori*.

Results Regular contact with animal stables before the age of 3 years (odds ratio (OR) (95% confidence interval): 2.0 [1.0; 4.0]) and unpasteurized milk consumption at age 6 years (1.8 [1.0; 3.3]) were the strongest risk factors for *T. gondii* infection. None of the farming-related factors were significantly associated with *H. pylori* infection. Current consumption of raw farm milk was not significantly associated with *H. pylori* infection (2.1 [0.8; 5.3]). Regular contact with animal houses before the age of 7 years was the strongest predictor for atopy (0.49 [0.26–0.96]). The reduction in risk could not be further decreased by any other factor under consideration. After adjustment for animal house contact, the OR for atopy was decreased by raw milk consumption and *H. pylori* infection in an additive manner.

Conclusion Exposure to farming environments in childhood might predict *T. gondii* seropositivity in rural subjects. Nevertheless, the strongest predictor for atopy in rural subjects seems to be regular contact with farm animals. Whether *T. gondii* infection is an intermediate factor in the association between farm contact and atopy needs to be confirmed in larger studies.

Keywords allergy, animal farming, asthma, atopy, epidemiology, field crop, *Helicobacter pylori*, hygiene hypothesis, *Toxoplasma gondii*, unpasteurized milk

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Introduction

Recent population-based studies in Italy [1], the US [2] and Denmark [3] have indicated that food-borne and orofecal infections may protect from asthma and allergies. This was especially shown for *Toxoplasma gondii* (*T. gondii*) [1–3], *Helicobacter pylori* (*H. pylori*) [1, 3–5] and Hepatitis A virus [1–3]. These results are in accordance with the 'hygiene hypothesis': Strachan postulated that as a result of an increase in T helper (Th)1 cell production associated with such infections, the immune system is skewed away from Th2 cell production and therefore, the prevalence of Th2 dominated diseases like atopic diseases may be decreased [6]. The observation that parasites associated with a Th2 dominated immune response, like hook worms, were also associated with a reduced prevalence of atopic diseases contradicted the hygiene hypothesis [6, 7]. The common

feature of worm infections and *T. gondii* as well as *H. pylori* infections is their chronicity. They produce a chronic persisting infection that usually persists indefinitely potentially even after specific treatment is provided [4, 8].

Other predictors inversely related to atopic diseases are factors related to exposure to farm animals. Studies from several countries across the world have shown a protective effect of being raised on a farm [9–11], exposure to animal stables during childhood [12–14], and being a farmer [15–17], on the prevalence of asthma and nasal allergies. Additionally, consumption of unpasteurized milk is inversely related to atopic diseases [13, 18].

While it has been shown that living on a farm is associated with a high overall burden of microbial compounds [12], the association between farming environments and markers of infection has not yet been assessed [19]. The source of *T. gondii* oocysts in the environment are cats. Once spread in the environment, sporulating oocysts might remain infectious for months to years [19]. The majority of transmission of *T. gondii* to humans is considered to be related to tissue cysts in infected meat and oocysts in food or water contaminated

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with feline feces [19]. The prevalence of antibodies to *T. gondii* is higher in rural areas than in urban areas [20, 21] and in farmers [22, 23]. An association between raw milk consumption and *T. gondii* infection has also been described [23].

It has been suggested that acquisition of *H. pylori* infection occurs in the first 5 years of life [8]. Therefore, *H. pylori* might be expected to have a particularly marked effect on the developing immune system [4]. Besides socio-economic status, the knowledge about potential risk factors for *H. pylori* infection is limited [24, 25]. Moreover, the mode of transmission of *H. pylori* still has to be clarified [24]. Besides person-to-person transmission, *H. pylori* might be transmitted via raw cow's [26] and sheep's milk [27]. No data on the prevalence of *T. gondii* and *H. pylori* infection do exist for the general population in Germany.

Thus, the aim of this study was to:

- Estimate the prevalence of *T. gondii* and *H. pylori* infection in young German adults living in rural environments.
- Investigate the association between factors associated with farming environments and *T. gondii* as well as *H. pylori* seropositivity.
- Evaluate the association between farming environment, markers of infection and atopy.

Methods

Source population

The study was nested in an ongoing cross-sectional study of asthma and allergies in young adults living in a rural part of Northern Germany with intensive livestock production. Within this study we followed the protocol of the European Community Respiratory Health Survey (ECRHS) [28]. All 4537 inhabitants, aged 18–44 years, living in a rural town in the study region were invited to answer a mail-in questionnaire. The questionnaire included items on respiratory and allergic diseases taken from the ECRHS questionnaire [28], as well as questions on exposure to farming environments [12, 13]. Up to two postal reminders were sent. Subjects not responding within 6 weeks after the first mailing were contacted by phone. Overall, 3112 (68.6%) subjects returned the completed questionnaire. The mean age (SD) of the 3112 participants was 33.0 (7.8) years as compared with 32.3 (7.7) years in the eligible population. The proportion of women among the participants was slightly higher than in the source population (51.2% vs. 48.2%, respectively).

Of those who answered the questionnaire, 930 (30%) were selected at random and invited to take part in the clinical examinations at the local study centre between April and August 2002. Of these, 520 subjects agreed to venous blood sampling (55.9%). With respect to respiratory symptoms and childhood contacts with farming environments, these subjects did not differ significantly from the rest of the source population. The study was approved by the local ethics committee.

Blood samples

After coagulation for 2 h the serum was separated by centrifugation at $1750 \times g$ for 10 min, stored at 3–5 °C for

up to 3 days and sent at 3–5 °C to Pharmacia, Freiburg using overnight service. The samples were stored at –20 °C until analysis.

IgE antibodies

Specific IgE against a panel of aeroallergens (birch pollen, mixed-grass pollen, mugwort pollen, dog dander, cat dander, *Cladosporium herbarum*, and *Dermatophagoides pteronyssinus*) were determined in serum using the Pharmacia CAP system (SX1; Pharmacia Diagnostics, Freiburg, Germany). Atopy was defined as any IgE antibody measurement in excess of 0.70 kU/L (class 2).

All subjects with IgE measurements in excess of 0.70 kU/L (class 2) ($n = 95$) and a random sample of 235 subjects with IgE results below 0.70 kU/L were re-contacted by phone in 2003 and additionally asked about raw milk consumption, cat and dog contact (response rate 100%). The interviewers were blinded to the atopy status of the participants. The serum samples of these subjects were additionally analysed for IgG against *T. gondii* and *H. pylori*.

IgG antibodies against *T. gondii* and *H. pylori*

Overall, 321 of the 330 serum samples could be analysed for IgG antibodies (97.3%). IgG specific for toxoplasma was determined by Enzygnost[®] Toxoplasmosis/IgG, and IgG specific for *H. pylori* was determined by Enzygnost[®] Anti-Helicobacter pylori II/IgG (both Dade Behring, Marburg, Germany). All assays were performed on a BEP III analyzer (Dade Behring) in a single laboratory, in parallel, according to the manufacturer's instruction. For *T. gondii* values ≥ 15 IU/mL and for *H. pylori* values > 10 U/mL were regarded as positive.

Definitions

The term 'farming-related factors' included: age at the start of regular contacts with animal stables (prior to kindergarten (before the age of 3 years); age while in kindergarten (between age 3 and 5 years); age at school (age 6–12 years)), and raw, uncooked farm milk consumption at school entry (age 6 years). Subjects reporting siblings or parents with atopic diseases (asthma, hayfever, or skin eczema) were considered to have a family history of atopy.

Statistical analysis

Cross-tabulation was used to visualize bivariate distributions of categorical predictors for atopic and non-atopic subjects. Additionally, the crude odds ratios (ORs) were calculated adjusting for age and gender. The potential associations between factors of farming and contact with pets inside home and markers of infections were analysed separately adjusting for age and gender. Additionally, a multivariate logistic regression model was developed in order to find the main farming-related predictors of *T. gondii* and *H. pylori* infections. However, living on a farm during the first 3 years of life was not included because it was highly correlated with age at the start of regular contacts with animal stables.

The associations between factors of farming environments, *T. gondii* as well as *H. pylori* infection and atopy were also

analysed using multiple logistic regression analyses. The following potential confounders were included: gender; age in years (18–26; 27–35; 36–44), smoking status; family history of atopy; number of siblings (<3 vs. ≥3 siblings); and currently living on a farm. Statistical analyses were carried out using SPSS statistical package.

Results

Descriptive data

Descriptive data are given in Table 1. Subjects with elevated IgE levels were more likely to be younger and to have less than three siblings. Having a family history of atopic diseases was strongly associated with atopy. With respect to factors related to farming, the proportion of atopics who had started regular visits to animal houses before the age of 7 years was significantly lower. Likewise, subjects with atopy were less likely to report raw milk consumption at school entry or to live on a farm during the first 3 years of life.

The prevalence of *T. gondii* and/or *H. pylori* seropositivity was 30.5%. Overall, 9.3% of the population had IgG against both *T. gondii* and *H. pylori*. *T. gondii* and *H. pylori* seropositivity were inversely related to being atopic; however, the association did not reach the level of statistical significance.

Predictors of *T. gondii* seropositivity

The associations between factors related to farming environments and *T. gondii* infection are given in Table 2. The major farming-related predictors of *T. gondii* infection were living on a farm during the first 3 years of life (OR [95% CI]: 3.0 [1.8–5.0]) and now (2.1 [1.1–3.9]), first regular contact with animal stables before the age of 3 years (2.9 [1.6–5.2]), and consumption of raw uncooked farm milk in childhood (2.2 [1.2–3.8]). In the multiple logistic regression model, the OR remained statistically significant for age at the start of regular contact with animal stables (Table 3). Raw milk consumption at school age was weakly associated with IgG against *T. gondii* (1.8 [1.0–3.3]). Age, gender, and level of education were not significantly associated with *T. gondii* infection.

Predictors of *H. pylori* seropositivity

H. pylori seropositivity increased significantly with age. Current consumption of raw farm milk and less than 12 years of schooling were associated with a non-significant increase in the risk of *H. pylori* seropositivity (Table 2). This was confirmed in the multiple logistic regression model including all parameters with borderline significance in the univariate analyses (Table 3).

Predictors of atopy

The association between markers of infection and atopy, as well as the association between farming-related factors and atopy, was evaluated in a multiple logistic regression model adjusting for potential confounders. The results are given as OR with 95% CI unadjusted for all factors listed in Table 4 and mutually adjusted for these parameters. Regular contact with animal houses before the age of 7 years was inversely

Table 1. Number (percentage) of cases and controls for several potential risk factors as well as OR (95% CI) for these parameters

	No atopy, N = 230 n (%)	Atopy, N = 91 n (%)	OR (95% CI)*
<i>Descriptives</i>			
<i>Age (years)</i>			
18–26	41 (17.8)	24 (26.4)	1
27–35	73 (31.7)	31 (34.1)	0.74 (0.38–1.43)
36–44	116 (50.4)	36 (39.6)	0.53 (0.28–1.00)
Gender: female	139 (60.4)	53 (58.2)	0.92 (0.56–1.52)
<i>No. of brothers and sisters</i>			
0	3 (1.3)	1 (1.1)	1
1–2	86 (37.6)	48 (53.9)	
≥3	139 (61.0)	40 (44.9)	
Kindergarten before the age of 5 years	67 (29.4)	37 (41.6)	1.70 (1.0–2.90)
≥12 years of schooling	45 (19.7)	23 (25.8)	1.31 (0.73–2.35)
<i>Family history of asthma/hayfever/eczema</i>			
Smoking	117 (50.9)	66 (72.5)	2.51 (1.47–4.30)
Smoking	72 (31.4)	24 (26.4)	0.73 (0.42–1.27)
<i>Respiratory symptoms</i>			
Symptoms of allergic rhinitis	13 (5.7)	54 (60.7)	
Doctors' diagnosed asthma	4 (1.8)	15 (16.5)	
<i>Farming-related risk factors</i>			
Farmer	13 (5.9)	3 (3.6)	0.62 (0.17–2.26)
Living on a farm now	39 (17.2)	12 (13.2)	0.68 (0.35–1.36)
Living on a farm during the first 3 years of life	106 (46.1)	27 (29.7)	0.54 (0.32–0.92)
<i>Age at the start of regular contact with animal houses</i>			
Never	120 (52.2)	61 (67.0)	1
>6	22 (9.6)	11 (12.1)	1.00 (0.45–2.23)
3–6	30 (13.0)	5 (5.5)	0.31 (0.11–0.85)
0–3	58 (25.2)	14 (15.4)	0.51 (0.26–0.99)
<i>Consumption of raw, uncooked farm milk</i>			
At age 6 years	136 (59.4)	35 (38.5)	0.46 (0.27–0.81)
Now	16 (7.0)	4 (4.9)	0.66 (0.21–2.06)
<i>Keeping pets at home</i>			
<i>Keeping a cat inside home</i>			
At age 6 years	30 (13.2)	9 (9.9)	0.72 (0.32–1.58)
Now	20 (8.7)	9 (9.9)	1.19 (0.52–2.76)
<i>Keeping a dog inside home</i>			
At age 6 years	51 (22.4)	14 (15.4)	0.58 (0.30–1.12)
Now	45 (19.6)	16 (17.6)	0.81 (0.43–1.54)
<i>Markers of infection</i>			
Neither <i>T. gondii</i> nor <i>H. pylori</i>	106 (46.1)	49 (53.8)	1
<i>T. gondii</i>	50 (21.7)	18 (19.8)	0.85 (0.44–1.62)
<i>H. pylori</i>	50 (21.7)	18 (19.8)	0.84 (0.44–1.61)
<i>T. gondii</i> and <i>H. pylori</i>	24 (10.4)	6 (6.6)	0.66 (0.25–1.80)

*Adjusted for age and gender. OR, odds ratio; CI, confidence interval; *T. gondii*, *Toxoplasma gondii*; *H. pylori*, *Helicobacter pylori*.

related to atopy. Neither markers of infection nor consumption of raw farm milk were significantly associated with atopy. The mutual adjustment affected the OR for *T. gondii* infection and raw milk consumption by more than 10% giving some indication for confounding by regular contact with animal houses in early childhood.

In order to evaluate which of the factors under consideration (farming exposure, markers of infection) is most important in the development of atopy, a sensitivity analysis was performed. The importance of early farm contact was

Table 2. Prevalence (*n* (%)) and OR (95% CI) of farming-related factors among subjects with and without IgG against *T. gondii* or *H. pylori*

<i>N</i> (%)	IgG against <i>T. gondii</i>			IgG against <i>H. pylori</i>		
	No (<i>n</i> = 223)	Yes (<i>n</i> = 98)	OR (95% CI)*	No (<i>n</i> = 223)	Yes (<i>n</i> = 98)	OR (95% CI)*
Farmer	7 (3.4)	9 (9.4)	2.73 (0.95–7.81)	11 (5.3)	5 (5.2)	0.97 (0.32–2.97)
Living on a farm now	28 (12.7)	23 (23.7)	2.08 (1.11–3.91)	36 (16.4)	15 (15.3)	0.84 (0.43–1.65)
Living on a farm during the first 3 years of life	72 (32.3)	61 (62.2)	3.01 (1.81–5.03)	91 (40.8)	42 (42.9)	0.86 (0.52–1.43)
Age at the start of regular contact with animal houses						
Never	135 (61.4)	44 (44.9)	1	124 (55.6)	57 (58.2)	1
Age (years)						
7–12	23 (10.3)	10 (10.2)	1.27 (0.55–2.95)	23 (10.3)	10 (10.2)	0.97 (0.42–2.23)
3–6	27 (12.1)	8 (8.2)	0.98 (0.41–2.37)	28 (12.6)	7 (7.1)	0.59 (0.24–1.46)
0–3	36 (16.1)	36 (36.7)	2.86 (1.59–5.15)	48 (21.5)	24 (24.5)	0.98 (0.54–1.77)
Consumption of raw, uncooked farm milk						
At age 6 years	103 (46.2)	68 (69.4)	2.15 (1.23–3.76)	115 (51.8)	56 (57.1)	0.92 (0.54–1.59)
Now	12 (5.4)	8 (8.2)	1.44 (0.56–3.75)	10 (4.5)	10 (10.2)	2.18 (0.86–5.55)
Keeping a cat inside home						
At age 6 years	24 (10.8)	15 (15.3)	1.61 (0.79–3.30)	30 (13.6)	9 (9.2)	0.63 (0.28–1.40)
Now	22 (9.9)	7 (7.1)	1.63 (0.66–4.05)	20 (9.0)	9 (9.2)	0.98 (0.42–2.30)
Keeping a dog inside home						
At age 6 years	45 (20.3)	20 (20.6)	1.16 (0.63–2.14)	47 (21.3)	18 (18.4)	0.97 (0.90–2.51)
Now	46 (20.6)	15 (15.3)	0.79 (0.41–1.53)	42 (18.8)	19 (19.4)	1.18 (0.63–2.20)

*Adjusted for age and gender. OR, odds ratio; CI, confidence interval; *T. gondii*, *Toxoplasma gondii*; *H. pylori*, *Helicobacter pylori*.

Table 3. Multiple logistic regression model: socio-demographic and farming-related factors as predictors and markers of infection as outcome

OR (95% CI)*, <i>N</i> = 300	<i>n</i>	<i>T. gondii</i>	<i>H. pylori</i>
Age (years)			
18–26	52	1	1
27–35	99	0.88 (0.37–2.07)	1.56 (0.70–3.46)
36–44	149	1.91 (0.87–4.22)	2.80 (1.34–5.86)
Gender: female	178	0.93 (0.54–1.60)	1.46 (0.87–2.44)
≥ 12 years of schooling	68	–	0.59 (0.31–1.15)
Farmer	16	2.02 (0.62–6.61)	–
Living on a farm now	48	1.26 (0.58–2.72)	–
Age at the start of regular contact with animal houses			
Never	164	1	–
Age (years)			
7–12	33	1.02 (0.43–2.43)	–
3–6	34	0.74 (0.29–1.85)	–
0–3	69	2.00 (1.01–3.96)	–
Consumption of raw, uncooked farm milk			
At age 6 years	166	1.80 (0.99–3.28)	–
Now	20	–	2.10 (0.83–5.35)
Keeping a cat inside home			–
at age 6 years	36	1.67 (0.77–3.62)	–

*OR mutually adjusted for all other factors included in the model. –, variable not included in the model. OR, odds ratio; CI, confidence interval; *T. gondii*, *Toxoplasma gondii*; *H. pylori*, *Helicobacter pylori*.

considered first in combination with the consumption of raw farm milk and IgG against *T. gondii* or *H. pylori*. As shown in Table 5, none of the other factors further decreased the association between early animal house contact and atopy. Therefore, early farm contact was included in the subsequent models evaluating the relative importance of raw milk consumption combined with markers of infection (Table 6). The OR for atopy decreased in an additive manner for the combination of raw milk consumption and *H. pylori* infec-

Table 4. Multiple logistic regression models using farming-related risk factors as well as markers of infection as predictors and atopy as outcome

		Atopy, <i>n</i> = 313	
	<i>n</i>	Crude OR (95% CI)*	Adjusted OR (95% CI)†
Markers of infection			
<i>T. gondii</i>	97	0.91 (0.51–1.64)	1.04 (0.56–1.91)
<i>H. pylori</i>	95	0.73 (0.41–1.31)	0.70 (0.39–1.28)
Age at the start of regular visits to animal houses			
> 6 years or never	207	1	1
≤ 6 years	106	0.43 (0.23–0.81)	0.49 (0.26–0.96)
Raw milk consumption			
No	144	1	1
Yes	169	0.53 (0.30–0.93)	0.65 (0.36–1.18)

*Adjusted for age, gender, smoking, living on a farm now, family history of atopy, number of siblings. †Adjusted for age, gender, smoking, living on a farm now, family history of atopy, number of siblings+mutually adjusted for all other parameters in the model. OR, odds ratio; CI, confidence interval; *T. gondii*, *Toxoplasma gondii*; *H. pylori*, *Helicobacter pylori*.

tion, i.e. subjects with IgG against *H. pylori* who consumed raw farm milk at the age of 6 years were least likely to be atopic (0.38 [0.15–0.98]). In contrast, no additional decrease in the risk estimates was seen for raw milk consumption combined with *T. gondii* seropositivity. A non-significant decrease in the relative odds was seen for the summary score of *T. gondii* and *H. pylori* seropositivity (Table 6).

Discussion

Our study is the first study in a rural German population estimating the prevalence of *T. gondii* and *H. pylori* infections. About one-third of the young adults living in a rural area were seropositive for *T. gondii* and *H. pylori*. In this

Table 5. Sensitivity analysis to evaluate the importance of each single factor in its effect on atopy using atopy as dependent variable and regular visits to animal stables before the age of 7 years combined with consumption of raw uncooked farm milk at the age of 6 years, and IgG against *T. gondii* or *H. pylori* as predictor variable

Atopy OR (95% CI)*	Age at the start of regular visits to animal houses			
	<i>n</i>	> 6 years or never	<i>n</i>	≤ 6 years
Model 1: raw milk consumption				
No	129	1	21	0.33 (0.10–1.09)
Yes	85	0.57 (0.29–1.11)	86	0.35 (0.17–0.74)
Model 2: IgG against <i>T. gondii</i>				
No	160	1	63	0.40 (0.18–0.88)
Yes	54	0.93 (0.45–1.92)	44	0.47 (0.19–1.15)
Model 3: IgG against <i>H. pylori</i>				
No	147	1	76	0.39 (0.19–0.80)
Yes	67	0.64 (0.32–1.27)	31	0.35 (0.12–1.04)

*Adjusted for age, gender, smoking, living on a farm now, family history of atopy, and number of siblings (<3 vs. ≥3). OR, odds ratio; CI, confidence interval; *T. gondii*, *Toxoplasma gondii*; *H. pylori*, *Helicobacter pylori*.

Table 6. Step 2 of the sensitivity analysis to evaluate the importance of each single factor in its effect on atopy using (A) raw milk consumption at the age of 6 years combined with *T. gondii* or *H. pylori* as predictor variables and (B) IgG against *H. pylori* combined with *T. gondii* as predictors

OR (95% CI)*	<i>N</i>	No	<i>n</i>	Yes
(A) Raw milk consumption				
Model 1: IgG against <i>T. gondii</i>				
No	120	1	103	0.65 (0.33–1.29)
Yes	30	1.06 (0.43–2.63)	68	0.68 (0.29–1.55)
Model 2: IgG against <i>H. pylori</i>				
No	108	1	115	0.78 (0.40–1.53)
Yes	42	0.95 (0.43–2.10)	56	0.38 (0.15–0.98)
(B) IgG against <i>H. pylori</i>				
Model 3: IgG against <i>T. gondii</i>				
No	151	1	65	0.73 (0.37–1.47)
Yes	67	1.04 (0.52–2.09)	30	0.64 (0.22–1.84)

*Adjusted for age, gender, smoking, living on a farm now, family history of atopy, number of siblings (<3 vs. ≥3), and regular contact with animal houses before the age of 7 years. OR, odds ratio; CI, confidence interval; *T. gondii*, *Toxoplasma gondii*; *H. pylori*, *Helicobacter pylori*.

population, farming-related factors were associated with an increased prevalence of *T. gondii* infection. In particular animal stable contact during early life and raw milk consumption predicted *T. gondii* seropositivity in young adulthood. In contrast, none of the farming-related factors were significantly associated with *H. pylori* seropositivity.

Adjustment for early farm exposure affected the risk estimates for *T. gondii* infection as well as raw milk consumption and atopy by more than 10% giving some indication that *T. gondii* infection might be an intermediate step in the observed association between animal house contact and atopy. However, as the association between IgG against *T. gondii* and atopy was only weak this could explain at most part of the farming effect. Studies on larger populations are needed to evaluate this finding in more detail.

Regular visits to animal houses in early childhood were the strongest predictor for atopy. This inverse association could

not be further decreased by any other factor under consideration. After adjustment for regular visits to animal houses in early childhood, the OR for atopy was decreased by *H. pylori* seropositivity and raw milk consumption in an additive manner. Therefore, these factors might act independently in the development of atopy.

Data on the prevalence of *T. gondii* seropositivity in the general population are rare [2, 20, 21]. However, our study does only reflect the prevalence in a rural population that might be considerably different from urban environments. To our knowledge, no data are available concerning the association between farming-related factors like regular contact with animal stables during childhood and *T. gondii* infection. We neither assessed raw meat consumption nor consumption of unwashed vegetable and fruits because we did not consider these factors as potential confounders in the association under study. All families involved in farming in our study were producing animals, 92% of them also produced field crops at their farms. Therefore, it might be concluded that children with regular contact with animal stables in early life also had contact with field crops and might have consumed unwashed, contaminated field crops. Cats, the source of *T. gondii* oocysts in the environment, can be found in the animal confinement house as well as on the fields. Therefore, whether the animal stable is the major source of *T. gondii* infection or whether it is a proxy for contact with fields cannot be evaluated from this study. It is interesting to note that especially animal stable contact at toddlers' age was associated with an increase in prevalence of *T. gondii* seropositivity. This might indicate that children at infant age are more likely to crawl on the ground of animal confinement buildings as well as crop fields and swallow soil from the ground. Especially in the farming environment, this dirt is more likely to contain infectious agents like *T. gondii* oocysts. However, to date no data on these potential associations are available. The association between raw milk consumption and *T. gondii* infection has been described earlier [23]. It might be that raw milk consumption also is a proxy for exposure to field crop and soil contaminated with oocysts. However, tachyzoites have been found in raw, uncooked milk [19]. Due to the lower concentration of proteolytic enzymes in the digestive tract, infants are considered to be at higher risk of transmission of *T. gondii* infection via raw farm milk [19].

Besides age, a non-significant association between current raw milk consumption and *H. pylori* seropositivity was seen. The number of subjects who currently consumed raw farm milk was low (*n* = 20). Additionally, these subjects might also differ from the rest of the population with respect to nutrition and/or other factors. However, as *H. pylori* has been found in unpasteurized farm milk [26, 27], it would be interesting to see whether such an association can also be found in children with and without regular raw milk consumption. The 30% reduction in the prevalence of atopy in subjects with IgG against *H. pylori* has also been found in other studies [1, 4], although the numbers in our study were not large enough to reach statistical significance.

Besides limited power, the major drawback of our study is the assessment of exposure to *T. gondii* and *H. pylori* as well as atopy at age 18–44 years, because we cannot assess the age at infection. This is especially important as it has been

hypothesized that the first year of life might be crucial for the development of atopic diseases. With respect to *H. pylori*, it has been suggested that the majority of infections occur during the first 5 years of life [8]. In contrast, our study showed an increased prevalence of *H. pylori* with age. This might indicate a cohort effect as well as a true age effect. Only birth cohort studies could provide data on the timing of exposure and outcome. Additionally, we cannot rule out recall bias in our study with respect to the childhood events under study. As it is not likely that this recall bias is differential, it most likely will lead to an underestimation of the association between exposure and disease [29]. Furthermore, farmers are a population that is less likely to report of respiratory symptoms as compared with the general population [15]. Therefore, we used specific IgE levels as outcome variable rather than symptoms of allergic rhinitis or asthma.

Our study implies that exposure to farming environments in early life may be associated with *T. gondii* infection. However, studies with a larger number of subjects, particularly children, are now warranted.

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