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Zoonotic helminth exposure and risk of allergic diseases: A study of two generations in Norway

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Summary

Background: Animal and human studies indicate that definitive host helminth infections may confer protection from allergies. However, zoonotic helminths, such as *Toxocara* species (spp.), have been associated with increased allergies.

Objective: We describe the prevalence of *Toxocara* spp. and *Ascaris* spp. seropositivity and associations with allergic diseases and sensitization, in 2 generations in Bergen, Norway.

Methods: Serum levels of total IgG4, anti-*Toxocara* spp. IgG4 and *Ascaris* spp. IgG4 were established by ELISA in 2 cohorts: parents born 1945-1972 (n = 171) and their offspring born 1969-2003 (n = 264). Allergic outcomes and covariates were recorded through interviews and clinical examinations including serum IgEs and skin prick tests.

Results: Anti-*Ascaris* spp. IgG4 was detected in 29.2% of parents and 10.3% of offspring, and anti-*Toxocara* spp. IgG4 in 17.5% and 8.0% of parents and offspring, respectively. Among offspring, anti-*Toxocara* spp. IgG4 was associated with pet keeping before age 15 (OR = 6.15; 95% CI = 1.37-27.5) and increasing BMI (1.16 [1.06-1.25] per kg/m^2). *Toxocara* spp. seropositivity was associated with wheeze (2.97[1.45- 7.76]), hayfever (4.03[1.63-9.95]), eczema (2.89[1.08-7.76]) and cat sensitization (5.65[1.92-16.6]) among offspring, but was not associated with allergic outcomes among parents. Adjustment for childhood or current pet keeping did not alter associations with allergies. Parental *Toxocara* spp. seropositivity was associated with increased offspring allergies following a sex-specific pattern.

Conclusions & Clinical Relevance: Zoonotic helminth exposure in Norway was less frequent in offspring than parents; however, *Toxocara* spp. seropositivity was associated with increased risk of allergic manifestations in the offspring generation, but not among parents. Changes in response to helminth exposure may provide insights into the increase in allergy incidence in affluent countries.

1 | INTRODUCTION

Exposure to micro-organisms may alter subsequent risk of non-infectious diseases, such as allergy. For example, protection against allergy as a result of being brought up on a farm[1] has been explained by the innate immune response being trained by the local microbial environment.[2] Conversely, pathogenic lower respiratory tract viral infections in early life, such as those caused by respiratory syncytial virus (RSV) infections, lead to a remodelling of the pulmonary immune system with potential elevated risk of subsequent allergic airway disease.[3]

It is hypothesized that exposure to helminths might be an important contributor to the risk of allergic disease. Helminth infections result in the host eliciting a type 2/Th2 immune response. This is characterized by helminth interaction with epithelial cells causing epithelial release of alarmin cytokines (eg, IL-25/IL-33) which drive innate lymphoid type 2 (ILC2) secretion of IL-4, IL-5 and IL-13. These cytokines support the induction of characteristic eosinophilia and M2 macrophage polarization along with CD4 T cell polarization to a Th2 phenotype and B cell secretion of IgE, a response similar to that which drives allergic pathology.[4] However, pre-clinical studies have demonstrated that helminth infections limit induction of type 2 allergic pathology by secreting substances that directly influence host immunity by, for example, raising regulatory immune response components (eg, regulatory T and B cells: Treg and Breg)[5,6] or limiting epithelial cell alarmin secretion.[7] Clinical studies in areas endemic for helminth infections support these findings.[8-10] The presence of helminth infection may be required for this protection as antihelminth therapy can lead to increased prevalence of allergic disease in helminth endemic regions.[11] These and other related studies have led to the suggestion that increasing and high rates of allergy in the developed world might be, at least in part, due to the loss of effective immune control by host-adapted parasitic helminth infections.[12]

However, helminth exposure may not always be protective against allergy. In high-income countries, the major human parasitic helminths associated with allergy protection have been controlled by strong public health provision breaking the cycle of infection.[13] However, zoonotic exposure is not uncommon through exposure to parasites of livestock and companion animals.[14-16] Indeed, a number of relatively recent studies have identified *Ascaris suum* infection and associated pathology (such as Loffler syndrome) to occur (albeit at low levels) in northern European and North American pig farming areas.[17-20] A more common cause of zoonotic helminth infections comes from *Toxocara canis and T. cati* which naturally infect dogs and cats.[21] According to recent studies, levels of human exposure to these parasites can vary from 6% to 87% depending on age and local environmental factors.[21-28] Zoonotic infection by *Toxocara* spp. is typically via ingestion of eggs by direct contact with dogs or cats or via fecally contaminated soil. Human infection by *Toxocara* spp. is, in most cases, asymptomatic.[21,22,29] However, a proportion of infections can provoke pathologies with rheumatic, neurologic and asthmatic symptoms.[23,30,31] Zoonotic *Toxocara* spp. exposure is also associated with increased risk of asthma and atopy.[23,25,29,32,33]

In this study, we aimed to address how common seroprevalence to *Ascaris* spp. and *Toxocara* spp. was in a Norwegian intergenerational cohort. Seropositivity to both parasites has been reported in northern Europe, but not in Scandinavia. Here, we established exposure by detecting the prevalence of circulating immunoglobulin G4 (IgG4) against both *Ascaris spp.* and *Toxocara* spp. by enzyme-linked immunosorbent assay (ELISA) in a Norwegian two-generation cohort. We then establish what associations existed between seropositivity to these parasites and allergic sensitization and diseases.

2 | METHODS

2.1 | Study population

This study is based on information and samples from Norwegian participants in 2 linked studies, the European Community Respiratory Health Survey (ECRHS; www.ecrhs.org)[34] and the Respiratory Health In Northern Europe, Spain and Australia study (RHINESSA; www.rhinessa.net).

The parent population comprised of 171 ECRHS participants born 1945-1972 from the study centre in Bergen (originally recruited from the general population aged 20-44 years in 1992-1994) that were followed up in 2010-2013. Participants underwent an interviewer-led questionnaire, lung function measurements and skin prick tests to aeroallergens, and provided blood samples for measurements

of total and aeroallergen-specific immunoglobulin Es (IgE) and serum parasite-specific IgG4s.

The offspring population included 264 adult and adolescent offspring (≥10 years of age, born 1969-2003) of the Bergen ECRHS participants. They were examined in 2014-2015 as part of the RHINESSA study, with questionnaires, clinical examination, skin prick tests and measurements of serum IgEs and IgG4s, following protocols comparable to those applied to the parents.

2.2 | Ethical approval

Approval was obtained from the Regional Committee for Medical and Health Research Ethics in Western Norway (approval numbers #2010/759 and #2012/1077). All participants provided informed written consent.

2.3 | Allergic sensitization and diseases

Allergic sensitization was determined by skin prick tests (SPT) to 12 allergens (ALK-Abello): timothy grass, ragweed, *Dermatophagoides pteronyssinus*, *D. farinea*, cat, dog, birch, *Blatella germanica*, olive, *Alternaria* spp., *Cladosporium* spp. *and Parietaria* spp., and 0.9% saline and 10 mg/mL histamine solution were used for negative and positive controls. Reactions to the allergens were read after 15 minutes. Reactivity was considered positive if the mean weal size was 3 mm greater than the negative control. Blood samples were collected and sera separated in SST Vacutainer glasses, centrifuged within 30-60 minutes after collection (at 2081 × g for 10 minutes, room temperature). The samples were stored at −20°C. Total IgE and specific IgE were performed according to standardized laboratory methods in Haukeland University Hospital in Bergen, Norway. IgE positivity was defined by IgE ≥ 0.35 kU/L to at least 1 of 4 allergens tested (cat, timothy grass and house dust mite).

Allergic diseases were assessed through standardized interviews, including questions on doctor's diagnosed asthma, symptoms of wheezing, hayfever (seasonal rhinitis), rhinitis (all year round) and eczema (see www.ecrhs.org and www.rhinessa.net for wording of questions).

2.4 | Preparation of helminth antigen

Toxocara canis worms were kindly provided by Professor Philip Cooper, Ecuador. *Ascaris lumbricoides* worms were obtained from Professor Mike Levin, Red Cross Children's Hospital, Cape Town. Whole worms were washed in distilled water with penicillin, streptomycin and fungizone to reduce contamination, and then washed 4 times with distilled water. Worms were then homogenized in filter-sterilized phosphate-buffered saline (PBS). The homogenate was centrifuged at 15 292 × g for 20 minutess and the soluble fraction collected and filtered through a 0.20-μm filter. Protein concentration of soluble worm antigen preparations was established using a bicinchoninic acid protein assay by Thermo Scientific (Rockford, IL, USA).

2.5 | Detection of total IgG4-, *Toxocara canis*- and *Ascaris lumbricoides*-specific antibodies by ELISA

Analysis of IgG4 towards *T. canis* and *A. lumbricoides* was performed for sera from both ECRHS3 and RHINESSA Bergen participants, whereas total IgG4 was quantified in sera from the RHINESSA participants only. Total IgG4 concentration was detected by ELISA using 96-well Nunc Immunosorb ELISA plates (Thermo Scientific) coated with 20 μg/mL of mouse monoclonal antibody against human IgG4 heavy chain in PBS. Participant plasma was diluted 1:20, 1:100, 1:500 and 1:2500 in PBS containing 1% bovine serum albumin (PBS-BSA). Serum antibody was detected using alkaline phosphatase-conjugated mouse anti-human IgG4 antibodies from Sigma-Aldrich (St. Louis, MI, USA). ELISA plates were read at 405 nm to determine optical density.

Detection of IgG4 antibodies against *T. canis and A. lumbricoides* was achieved using an indirect ELISA. Ninety-six-well Nunc Immunosorb ELISA plates (Thermo Scientific) were coated with 10 μg/mL of soluble helminth antigen diluted in carbonate buffer. The serum from participants was diluted 1:20, 1:100, 1:500 and 1:2500 in PBS-BSA. Bound antibodies were detected using alkaline phosphatase-conjugated mouse anti-human IgG4 antibodies (Sigma-Aldrich). ELISA plates were read at 405 nm to determine optical density (OD). Relative plasma recognition of soluble worm antigen was calculated from optical density vs sample dilution curve.[35]

Antihelminth immunoglobulin responses can be cross-reactive between helminth species.[36,37] Anti-*A.lumbricoides* IgG4 (anti-*Ascaris* IgG4) was used as a general marker of exposure to parasitic nematodes. To reduce cross-reactivity in assessment of *Toxocara*, sera were pre-incubated on *A. lumbricoides* antigen-coated plates and then transferred to *Toxocara* antigen-coated plates.

2.6 | Covariates

Data relating to age, sex, education level, smoking status, parental history of allergic disease, place of upbringing (farm with livestock, farm without livestock, village in rural area, small town, suburb of city, inner city) and pet ownership (cats and dogs in childhood and current pet keeping) were retrieved from interviews performed during the clinical examinations of the ECRHS and RHINESSA participants, the same day as the blood samples were taken. Smoking (in adults) was categorized into never smokers, previous smokers and current smokers. The study subject's level of education was categorized as primary school, secondary/technical education and college/university, whereas the adolescents were categorized as being students/still in school.

2.7 | Statistical analyses

Descriptive statistics for the study population were reported as mean and range or standard deviation for continuous variables and count and percentages for categorical variables.

Logistic regression was applied to assess associations between pet keeping, place of upbringing, age and sex as well as other

potential variables that might be associated with helminth exposure (anti-*Toxocara* and anti-*Ascaris* IgG4 positivity). Similar models were applied to study associations between *Toxocara* seropositivity and allergic sensitization (specific IgE and SPT towards inhalant allergens), total IgE, rhinitis, hayfever and asthma. Models were performed separately or combined for the 2 generations (ECRHS parent and RHINESSA offspring generations). In all regression models with the combined study populations and when analyzing the offspring generation, we corrected for clustering within families (parent-offspring and/or siblings) by applying a cluster for family-id. To discriminate between helminth infections that can translate into high specific IgE which does not necessarily reflect SPT reactivity, we performed sensitivity analyses with separate models for *Toxocara* and *Ascaris* IgG4 sero positivity and associations with SPT and specific IgE towards any inhalant, cat, HDM and timothy (grass) allergens.

STATA (StataCorp, College Station, TX, USA), version IC 14.0, was used in all analyses.

3 | RESULTS

3.1 | Characteristics of study populations

The median age was 26 years in the offspring generation and 53 years in the parent generation (Table 1). The education level in this population was high, with more than 50% of study participants reporting University or college degrees. The parent generation had more often kept cats in childhood, but there was no statistical difference in dog keeping between the 2 generations (Table 1). Current pet keeping was similar between the 2 cohorts. Of the parent generation, 15% had grown up on a farm with livestock, compared to only 0.8% of the offspring generation. The 2 generations had similar lifetime prevalence of rhinitis and hayfever, but the offspring generation had a higher prevalence of positive allergy test (positive SPT or IgE towards at least 1 inhalant allergens) than the parent generation (44.5% vs 31.1%, respectively, $P = .006$) (Table 1).

3.2 | Detection of IgG4 to helminth antigens

Overall, 11.7% had detectable levels of anti-*Toxocara* spp. IgG4, with a higher prevalence among the parents (17.5%) than among the offspring (8.0%), $P = .002$ (Table 1). Overall, 17.9% of the study population had detectable levels of anti-*Ascaris* spp. IgG4; 29.2% in the parent generation and 10.3% in the offspring generation. Among the participants with elevated levels of *Toxocara* spp. IgG4, 88% also had elevated levels of anti-*Ascaris* spp. IgG4, suggesting sera cross-reactivity and/or simultaneous exposure to other parasitic nematodes. The seroprevalence of *Toxocara* and *Ascaris* IgG4 was decreasing in more recent cohorts (Figures 1 and 2) with a statistically significant trend for *Toxocara* in offspring (Figure 1) and decreasing trend for *Ascaris* for parent and offspring combined ($P = .07$, Figure 2). Among the offspring, we also detected total IgG4 in 77% and 86% of the subjects with either anti-*Ascaris* spp. or anti-*Toxocara* spp. IgG4, respectively. Total IgG4 was associated with cat and dog keeping in childhood.

3.3 | Factors associated with exposure to helminths

Seropositivity to *Toxocara* spp. and *Ascaris* spp. increased with age and with BMI among the offspring, but did not differ significantly by gender (Table 2A and B). *Toxocara* spp. seropositivity was associated with pet keeping before the age of 15 among the offspring (OR = 6.15 [1.37-27.54], $P = .02$), but was not associated with pet keeping among the parents (Table 2A). For *Ascaris* spp. seropositivity, associations were seen for cat keeping during both early and late childhood in the parent generation (Table 2B). Current pet ownership was not associated with raised anti-*Toxocara* spp. IgG4 or anti-*Ascaris* spp. IgG4. The risk of *Ascaris* seropositivity was increased among parents who had grown up on a farm with livestock (OR = 3.38 [1.31-8.69]) or in a small town (OR = 2.36 [1.06-5.27], $P = .04$ for trend) as compared to those growing up in the city or in the suburbs (Table 2B). There was no association between *Toxocara* spp. and *Ascaris* spp. seropositivity and total IgE (data not given).

3.4 | Exposure to helminths as associated with risk of allergic sensitization and diseases

In the offspring generation, anti-*Toxocara* IgG4 was associated with 3 to 4 times increased risk of reported wheeze, hayfever and eczema (Table 3A). Anti-*Toxocara* IgG4 was further associated with increased risk of positive SPT/IgE towards cat allergens (OR = 5.65 [1.92-16.6]). In the parent generation, anti-*Toxocara* IgG4 was not significantly associated with any allergic outcome; the associations were generally in a negative direction (Table 3A). For anti-*Ascaris* IgG4, the patterns were generally similar, with anti-*Ascaris* IgG4 being associated with increased allergic outcomes in the offspring generation but not in the parent generation (Table 3B). In the model assessing cat allergy, we tested for interaction between *Toxocara* seropositivity and childhood cat keeping. No significant interaction was found ($P = .34$). The associations between allergies and *Toxocara* seropositivity were not altered when adjusted for childhood or current pet keeping. The results of the sensitivity analyses for *Toxocara* spp. and *Ascaris* spp. with separate models for specific IgE and SPT results differed from the models using a combined measure for specific IgE and SPT. Positive results were seen for offspring *Toxocara* spp. positivity and both SPT and IgE towards timothy (aOR = 2.91 [95% CI: 1.11, 7.63] and 3.36 [1.24, 8.44], respectively) (Table S1). *Ascaris* spp. positivity was borderline significant for SPT towards cat (aOR = 2.67 [0.99, 7.24], $P = .05$), but non-significant for *Ascaris* spp. and cat-specific IgE (Table S2).

Although parents' *Toxocara* seropositivity was not associated with their own allergic manifestations, parents' *Toxocara* seropositivity appeared to be associated with allergic manifestations in their offspring (Table 4). Gender-specific patterns, indicating associations between paternal exposure and their daughters' outcomes (significant for asthma, eczema and timothy grass) and maternal exposure and their sons' outcomes (significant for any specific IgE positivity and sensitization to cat allergens), were also found (Table 4).

TABLE 1 Characteristics of study populations (in percent unless otherwise specified)

	All (n = 435)	Parents (n = 171)	Offspring (n = 264)	P-value
Gender (% men)	53.9	52.6	54.8	.7
Age in years (median (range))	35 (10-63)	53 (39-63)	26 (10-45)	
BMI (kg/m^2) median (range)		25.6 (18.8-42.0)	24.6 (15.1-40.1)	
Education level				
Student/adolescent	6.3		10.8	
Primary	4.3	8.2	1.6	
Secondary	38.0	43.5	34.2	
University or college	51.4	48.2	53.7	.003[a]
Cat ownership				
Early childhood	22.8	30.6	17.7	.003
Late childhood	43.0	46.8	40.6	.2
Current	21.6	21.3	21.7	.9
Dog ownership				
Early childhood	11.8	8.9	13.7	.2
Late childhood	28.6	27.6	29.2	.7
Current	18.2	18.3	18.0	.9
Place of upbringing				
City or suburb	57.8	46.2	65.8	
Small town	16.6	28.1	8.6	
Village in rural area	15.4	3.5	23.8	
Farm without livestock	3.6	7.0	1.2	
Farm with livestock	6.8	15.2	0.8	<.001
Smoking status				
Never	55.3	40.6	66.8	
Previous smoker	28.9	38.2	21.7	
Current smoker	15.8	21.2	11.5	<.001*
Parental asthma	14.7	7.7	19.8	
Parental asthma not known	8.2	15.4	3.0	.004
Wheeze, ever	18.3	24.7	13.9	.005
Hayfever, ever	24.2	25.4	23.4	.6
Rhinitis, ever	49.8	51.2	48.9	.6
Asthma, ever	14.2	14.1	14.2	.97
Eczema, ever	43.7	49.4	40.0	.06
Any positive allergy test (specific IgE or SPT)[b]	39.3	31.1	44.5	.006
IgE positivity[c]	32.7	19.5	41.2	<.001
Total IgE in kU/L (mean (SD))	71.6 (193.9)	69.9 (240.9)	72.9 (156.7)	.6
Total IgG4 (% positive)[d]			11.4	
Ascaris IgG4 (% positive)	17.9	29.2	10.3	<.001
Toxocara IgG4 (% positive)	11.7	17.5	8.0	.002

[a]Does not include students.
[b]Positive IgE ≥ 0.35 kU/L and/or skin prick test positivity towards at least 1 of 4 allergens (cat, timothy grass, birch and house dust mite).
[c]Positive IgE ≥ 0.35 kU/L to at least 1 of 4 allergens tested (cat, timothy grass, birch and house dust mite).
[d]Only measured for offspring. Information missing for sex and age (n = 3), BMI (n = 3 for offspring, n = 7 for parents), education level (n = 19), father/mother asthma (n = 34) place of upbringing (n = 20), cat ownership (early childhood: n = 41, late childhood: n = 35, current: n = 22), dog ownership (early childhood: n = 44, late childhood: n = 36, current: n = 22), wheeze (n = 20), hayfever, rhinitis, asthma, positive allergy test (n = 5), eczema (n = 7), total and specific IgE (n = 4), *Ascaris* (n = 11),* smoking status only available for adults. Parents=ECRHS. Offspring=RHINESSA.
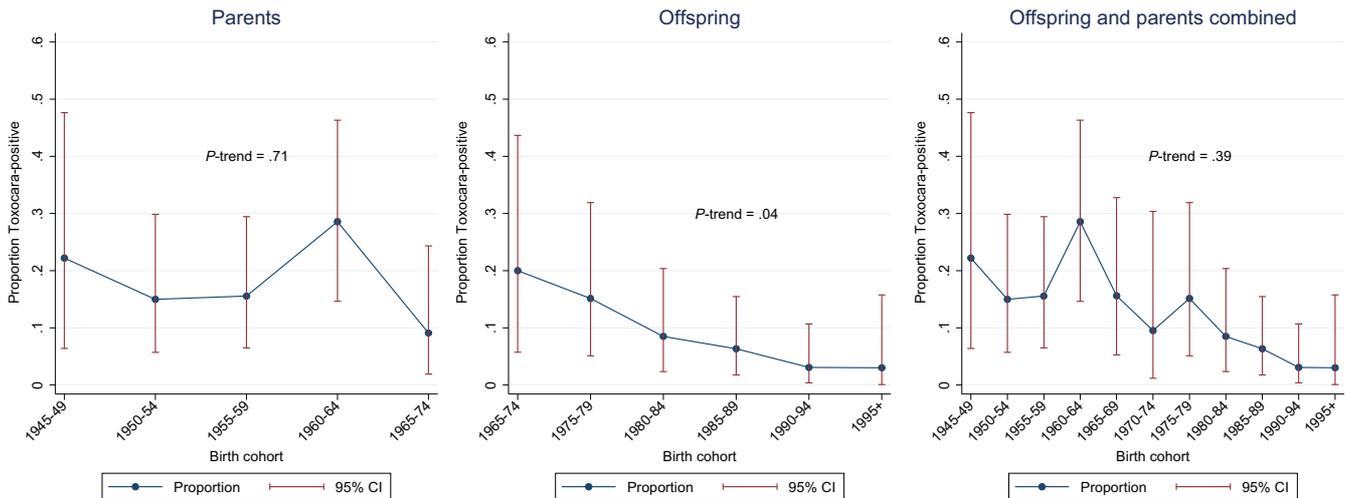
FIGURE 1 Anti-*Toxocara* seropositivity according to birth cohort in the parent generation, the offspring generation and in both combined
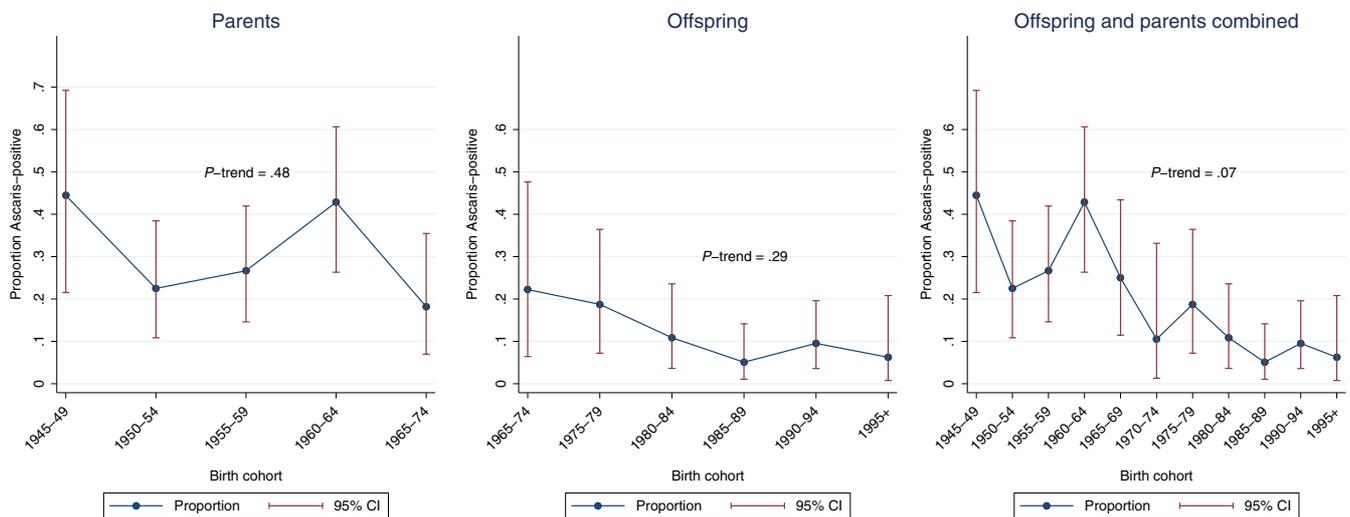

FIGURE 2 Anti-*Ascaris* seropositivity according to birth cohort in the parent generation, the offspring generation and in both combined

4 | DISCUSSION

In this study, we present evidence that exposure to helminths exists in a Norwegian population and that this parasitic exposure may have important health implications. To the best of our knowledge, this is the first study to address prevalence of zoonotic exposure to helminths in Norway. We detected antibodies against *Toxocara* spp. and *Ascaris* spp. in 12% and 18% of the study participants, respectively. We found a higher prevalence of exposure in participants born between 1945 and 1972 (parents) compared to participants born between 1969 and 2003 (offspring).

Helminth exposure was associated with childhood cat keeping in both the parents and offspring generations, place of upbringing in parents, and increasing age and increasing BMI in offspring. Exposure to *Toxocara* spp. was an important risk factor for allergic disease. In the offspring generation, *Toxocara* spp. seropositivity was associated with a 3-4 times increased risk of several allergic

outcomes. However, in the parent generation, *Toxocara* spp. seropositivity was not associated with parental allergic outcomes, but did associate with allergic outcomes in their offspring following a complex sex-specific pattern. Neither childhood nor current pet keeping explained the associations of *Toxocara* spp. with allergies.

The 12% prevalence of anti-*Toxocara* IgG in our study corresponds with reports from other European countries and the United States, with 8%-11% reported for Dutch children,[32,33] 8% for Dutch adults,[14] 14% in a US population-based study (NHANES),[28] but lower than among Spanish adults (29%).[29] We found that *Toxocara* spp. seropositivity was associated with allergic diseases and sensitization among the offspring. This is in agreement with findings reported from other regions; for example, Mughini-Gras et al[14] reported an association of anti-*Toxocara* IgGs with hayfever in the Netherlands. Higher prevalence of exposure to *Toxocara* spp. has also been reported in atopic populations in low to middle income countries including Malaysia (21% among asthmatics and 9% in non-

TABLE 2 Odds ratio (OR) and 95% CI for (A) anti-*Toxocara* spp., (B) anti-*Ascaris* spp. IgG4 positivity and associations with gender, age, BMI, cat and dog keeping and place of upbringing

	Parents (n = 171)		Offspring (n = 264)	
	OR (95% CI)	*P*-value	OR[a] (95% CI)	*P*-value
(A)				
Gender (ref men)	0.69 (0.31, 1.55)	.4	0.63 (0.26, 1.51)	.3
Age	1.06 (0.99, 1.14)	.7	**1.10 (1.03, 1.17)**	**.009**
BMI (per kg/m2)	0.98 (0.88, 1.09)	.7	**1.16 (1.06, 1.25)**	**<.001**
Cat keeping				
Early childhood	1.42 (0.60, 3.39)	.3	1.94 (0.61, 6.19)	.2
Late childhood	1.53 (0.66, 3.52)	.3	**6.25 (1.94, 20.12)**	**.002**
Current	0.51 (0.17, 1.58)	.3	0.70 (0.22, 2.22)	.5
Dog ownership				
Early childhood	1.35 (0.35, 5.22)	.9	1.50 (0.39, 5.76)	.5
Late childhood	0.90 (0.35, 2.32)	.8	1.63 (0.57, 4.68)	.4
Current	0.44 (0.13, 1.56)	.2	0.86 (0.29, 2.62)	.8
Any pets from birth to age 15	1.79 (0.70, 4.53)	.2	**6.15 (1.37, 27.54)**	**.02**
Place of upbringing				
City or suburb (ref)		.06		.6
Small town	1.05 (0.38, 2.94)		1.18 (0.24, 5.89)	
Village in rural area	3.09 (0.50, 18.9)		0.83 (0.24, 2.94)	
Farm without livestock	1.24 (0.24, 6.41)		-	
Farm with livestock	2.75 (0.96, 7.84)		-	
(B)				
Gender (ref men)	0.83 (0.43, 1.60)	.6	0.55 (0.25, 1.21)	.2
Age	1.07 (1.01, 1.13)	.5	1.06 (1.00, 1.12)	.08
BMI (per kg/m2)	0.99 (0.91, 1.09)	.9	**1.17 (1.08, 1.26)**	**<.001**
Cat keeping				
Early childhood	**2.32 (1.11, 4.84)**	**.04**	1.21 (0.40, 3.65)	.6
Late childhood	**2.02 (0.99, 4.15)**	**.06**	1.76 (0.69, 4.44)	.2
Current	0.52 (0.21, 1.29)	.2	0.72 (0.25, 2.07)	.5
Dog ownership				
Early childhood	1.54 (0.48, 4.89)	.8	0.79 (0.16, 3.76)	.8
Late childhood	0.77 (0.34, 1.74)	.5	1.62 (0.63, 4.15)	.3
Current	0.41 (0.15, 1.14)	.09	1.91 (0.80, 4.58)	.1
Any pets from birth to age 15	**2.75 (1.21, 6.25)**	**.02**	1.94 (0.70, 5.35)	.2
Place of upbringing				
City or suburb (ref)		.04		.4
Small town	**2.36 (1.06, 5.27)**		0.82 (0.17, 4.05)	
Village in rural area	1.97 (0.33, 11.72)		0.72 (0.23, 2.29)	
Farm without livestock	0.78 (0.16, 3.96)		-	
Farm with livestock	**3.38 (1.31, 8.69)**		-	

[a]Corrected for clustering within families. Parents=ECRHS; Offspring=RHINESSA. Statistically significant odds ratios highlighted in bold.

asthmatics),[30] Turkey (13% of asthmatics and 2% of non-asthmatics)[31] and Sri Lanka (29% of asthmatics and 10% in non-asthmatics).[23] Why zoonotic *Toxocara* spp. infections do not confer the protection associated with natural helminth infections is not known, but may possibly be due to *Toxocara* spp.-secreted immune regulatory products being active against dog and cat immune responses and not against human.[38] Therefore, the *Toxocara* spp. induced type 2 immune response may lack effective regulation in a human host, thereby promoting allergic pathology and potentially sensitization to other allergens.[39]

TABLE 3 Adjusted odds ratio (aOR) and 95% CI for (A) anti-*Toxocara* spp. IgG4 (B) anti-*Ascaris* spp. IgG4 positivity and associations with respiratory symptoms and allergic sensitization

	Parents (n = 171)		Offspring (n = 264)	
	aOR[a] (95% CI)	*P*-value	aOR[a] (95% CI)	*P*-value
(A)				
Wheeze, ever	0.77 (0.28, 2.08)	.6	**2.97 (1.45, 7.76)**	.03
Asthma, ever	0.86 (0.27, 2.77)	.8	1.24 (0.29, 5.19)	.7
Hay fever, ever	0.97 (0.38, 2.49)	1.0	**4.03 (1.63, 9.95)**	.003
Rhinitis, ever	0.61 (0.27, 1.37)	.2	3.06 (0.97, 9.72)	.06
Eczema, ever	1.02 (0.45, 2.32)	1.0	**2.89 (1.08, 7.76)**	.04
Any positive allergy test[b]	0.56 (0.21, 1.48)	.2	1.22 (0.52, 2.88)	.6
Any IgE-positive[c]	0.76 (0.26, 2.19)	.6	1.84 (0.78, 4.38)	.2
Cat SPT/IgE-positive[d]	0.72 (0.15, 3.46)	.7	**5.65 (1.92, 16.6)**	.002
HDM SPT/IgE-positive[e]	0.46 (0.10, 2.13)	.3	1.41 (0.52, 3.81)	.5
Timothy grass SPT/IgE-positive[f]	1.00 (0.31, 3.24)	1.0	2.12 (0.85, 5.33)	.1
(B)				
Wheeze, ever	0.72 (0.31, 1.64)	.4	1.81 (0.70, 4.68)	.2
Asthma, ever	0.94 (0.36, 2.46)	.9	0.47 (0.10, 2.25)	.3
Hay fever, ever	1.60 (0.75, 3.41)	.2	**3.50 (1.42, 8.63)**	.007
Rhinitis, ever	0.87 (0.45, 1.71)	.7	2.11 (0.82, 5.40)	.1
Eczema, ever	0.83 (0.42, 1.65)	.6	2.06 (0.85, 5.98)	.1
Any positive allergy test[b]	0.80 (0.38, 1.70)	.6	1.16 (0.50, 2.69)	.7
Any IgE-positive[c]	0.45 (0.17, 1.18)	.1	1.66 (0.71, 3.86)	.2
Cat SPT/IgE-positive[d]	0.62 (0.16, 2.33)	.5	**3.31 (1.19, 9.17)**	.02
HDM SPT/IgE-positive[e]	0.75 (0.26, 2.19)	.6	1.29 (0.48, 3.49)	.6
Timothy grass SPT/IgE-positive[f]	1.32 (0.52, 3.39)	.6	**2.66 (1.10, 6.47)**	.03

[a]Adjusted for: gender, age, BMI and corrected for clustering within families.
[b]Positive IgE ≥ 0.35 kU/L and/or skin prick test positivity towards at least 1 of 4 allergens (cat, timothy grass, birch and house dust mite).
[c]Positive IgE ≥ 0.35 kU/L to at least 1 of 4 allergens tested (cat, timothy grass, birch and house dust mite).
[d]Positive IgE ≥ 0.35 kU/L and/or skin prick test positivity towards cat allergen.
[e]Positive IgE ≥ 0.35 kU/L and/or skin prick test positivity towards house dust mite (*D. pteronyssinus* and/or *farinae*).
[f]Positive IgE ≥ 0.35 kU/L and/or skin prick test positivity towards timothy grass. Parents=ECRHS. Offspring=RHINESSA. Statistically significant odds ratios highlighted in bold

An unexpected finding from our study was the large differences in allergic risk association between the parent and offspring generations. No effects from *Toxocara* spp. seropositivity were indicated in the parent generation. However, *Toxocara* spp. seropositivity was strongly associated with increased risk of allergic outcomes in the offspring generation. This may imply that early-life exposure to *Toxocara* is a risk factor for development of allergic disease and that the risk resulting from this exposure may not be lifelong. Changes in microbial diversity in the environment might potentially be of importance for response to helminths, as it has been demonstrated that gut microbiota can alter the response to *Toxocara* in mice.[40]

The age effect in our study, with *Toxocara* seroprevalence increasing with age in the offspring generation, might reflect a time trend rather than a biological age patterns, and decreasing seroprevalence of *Toxocara* in more recent birth cohorts is presented in Figure 1. Similar trends were observed for *Ascaris*. Comparison of offspring and parents in the present study indicated that both *Toxocara* and *Ascaris* exposure were lower in offspring. Rural areas tend to exhibit higher prevalence of human exposure to helminths (35%-42%) than semi-rural (15%-20%) or urban (2%-5%) areas.[41] Although urban living is the most fitting description of the populations in the present study, urban areas, such as parks and town squares, can contain high numbers of *Toxocara* eggs and may represent a substantial risk factor for infection by *Toxocara* spp. Indeed, some studies have reported higher levels of *Toxocara* seroprevalence among subjects living in urban compared to rural areas.[27,29]

An intriguing finding in our study was the association of parental *Toxocara* spp. seropositivity with offspring allergic outcomes, namely paternal exposure increased risk of allergy in daughters and maternal exposure increased risk in sons. Adjustment for pet keeping did not alter the associations; thus, shared environment did not appear to explain the findings. In addition, adjustment for parental allergies did not alter the findings, suggesting that heritability in allergy or reverse causation did not explain the findings. Others have demonstrated maternal helminth exposure to influence offspring susceptibility to allergy,[42] and there are mice models demonstrating that maternal

TABLE 4 Adjusted odds ratio (aOR) and 95% CI for parental anti-*Toxocara* spp. IgG4 positivity and associations with respiratory symptoms and allergic sensitization in offspring

	All offspring (n = 264) aOR[a] (95% CI)	Paternal line		Maternal line	
		Sons (n = 71) aOR[b] (95% CI)	Daughters (n = 69) aOR[b] (95% CI)	Sons (n = 69) aOR[b] (95% CI)	Daughters (n = 48) aOR[b] (95% CI)
Wheeze, ever	1.99 (0.54, 7.34)	0.95 (0.11, 8.04)	0.66 (0.08, 5.49)	6.93 (0.50, 95.2)	-
Asthma, ever	**2.85 (1.16, 7.00)**	0.57 (0.06, 5.14)	**5.43 (1.29, 22.9)**	4.96 (0.96, 25.6)	-
Hay fever, ever	1.66 (0.77, 3.61)	0.69 (0.17, 2.71)	2.26 (0.68, 7.44)	3.91 (0.98, 15.67)	0.91 (0.10, 8.62)
Rhinitis, ever	1.47 (0.68, 3.19)	0.62 (0.16, 2.39)	1.78 (0.52, 6.01)	1.95 (0.54, 7.06)	0.72 (0.08, 6.21)
Eczema, ever	1.94 (0.93, 4.04)	1.75 (0.51, 6.03)	**3.89 (1.18, 12.8)**	0.68 (0.12, 3.89)	2.76 (0.28, 27.5)
Any positive allergy test[c]	1.92 (0.90, 4.09)	1.41 (0.35, 5.65)	2.21 (0.74, 6.64)	4.42 (0.96, 20.29)	0.86 (0.16, 4.62)
Any IgE-positive[d]	1.13 (0.50, 2.56)	0.99 (0.25, 3.95)	1.26 (0.36, 4.36)	**6.74 (1.52, 29.9)**	0.44 (0.07, 2.99)
Cat SPT/IgE-positive[e]	1.50 (0.53, 4.20)	1.24 (0.25, 6.10)	1.58 (0.29, 8.64)	**5.40 (1.22, 23.8)**	0.77 (0.10, 5.77)
HDM SPT/IgE-positive[f]	1.31 (0.51, 3.40)	2.21 (0.52, 9.38)	1.09 (0.26, 4.46)	1.56 (0.43, 5.65)	0.54 (0.07, 4.37)
Timothy grass SPT/IgE-positive[g]	**2.27 (1.12, 4.60)**	1.32 (0.37, 4.76)	**3.82 (1.21, 12.0)**	2.12 (0.52, 8.62)	1.75 (0.31, 9.81)

[a]Adjusted for: offspring age, parent age, offspring gender, parent gender, offspring *Toxocara* seropositivity, offspring education, offspring BMI, parental asthma/allergy, and corrected for clustering within families.
[b]Adjusted for: offspring age, parent age.
[c]Positive IgE ≥ 0.35 kU/L and/or skin prick test positivity towards at least 1 of 4 allergens (cat, timothy grass, birch and house dust mite), also associated with parental allergy.
[d]Positive IgE ≥ 0.35 kU/L to at least 1 of 4 allergens tested (cat, timothy grass, birch and house dust mite).
[e]Positive IgE ≥ 0.35 kU/L and/or skin prick test positivity towards cat allergen.
[f]Positive IgE ≥ 0.35 kU/L and/or skin prick test positivity towards house dust mite (*D. pteronyssinus* and/or *farinae*).
[g]Positive IgE ≥ 0.35 kU/L and/or skin prick test positivity towards timothy grass. Parents=ECRHS. Offspring=RHINESSA. Gender missing for 3 offspring. Statistically significant odds ratios highlighted in bold.

helminth infection influences immunological characteristics in pups.[43] With regard to differential risk outcome by gender, research focused on other exposures than helminths has found that parental pre-conception exposure might influence disease risk in offspring differently through the maternal and paternal lines.[44,45] The risk of allergy has been reported not only to be affected by the maternal or paternal line, but also to depend on the sex of the child.[44,46,47] In our study, parental anti-*Toxocara* IgG4 was measured years after the offspring were born. The half-life of IgG4 in general is approximately 21 days,[48] but *Toxocara* larvae may stay capsulated in tissues for many years in accidental hosts.[49] Our findings may suggest either ongoing antigenic exposure or the existence of long-lived memory/plasma B cell populations as a source of this IgG4. The mechanisms that may underlie the parental effect on offspring are beyond the scope of our current study. The effect may be due to any range of potential parental influences on the offspring including (but not limited to) post-translational modification,[50] shared environment,[51] microchimerism[52] or passive transfer of immune markers or antibodies.[53,54]

In the present study, we also addressed *Ascaris* seroprevalence in the cohort and found higher rates of seropositivity in the parent (29%) than in the offspring generation (10%). *Ascaris* spp. exposure could be acquired via a number of sources, most plausibly via exposure to agricultural or wildlife sources of *Ascaris* spp. (especially *A. suum*), which are recognized zoonoses in northern Europe.[17,20] That *Ascaris* spp. IgG4 was also more commonly detected in individuals associated with exposure to livestock farm supports this

hypothesis. Less likely may be the maintenance of *A. lumbricodes* infection cycle in the study area. The data may also reflect non-specific ELISA reaction due to antigen cross-reactivity. Helminth proteins targeted by IgE have been shown to be cross-reactive with other allergens which include proteins from house dust mite and cockroach,[55] and cross-reactivity between *Ascaris lumbricoides* and *Dermatophagoides farinae* IgG has been demonstrated in rabbits.[56] However, as documented cases of toxocariasis are rarely described in Norway, we would not expect our study participants to have specific IgE towards *Toxocara* spp. We therefore focused on assessing IgG4 towards *Toxocara* spp., which is most commonly used for serodiagnosis of toxocariasis.[57] Although we did not have data on specific IgE towards *Ascaris* or *Toxocara* in the present study, we cannot exclude the possibility that exposure to HDM or cockroach allergens may have influenced *A. lumbricoides* IgG4 seropositivity as observed in the present study. However, we did not observe any association between *Ascaris* or *Toxocara* IgG4 and allergic sensitization (SPT or specific IgE) towards HDM, which is the most commonly described cross-reactive allergen with helminths. Anti-*Ascaris* antibodies are also recognized as being cross-reactive to other helminth antigens[58]; in particular, species within the group of ascaridoid nematodes, such as *A. lumbricoides*, *A. suum* and *T. canis*, show antigenic relationship.[36] Thus, detection of anti-*Ascaris* IgG4 may reflect exposure to other helminths or allergens. Similarly, our use of somatic *Toxocara canis* antigen as opposed to excretory-secretory *Toxocara canis* antigen may have reduced the specificity and sensitivity of our ELISA. However, somatic *Toxocara* spp. antigen ELISA is

acceptable for detecting exposure to *Toxocara* spp.[57] Moreover, we based our definition of exposure on elevated levels of *Toxocara* spp. IgG4 which lends greater specificity[59] to our findings and therefore greater confidence that our measurements genuinely reflect exposure to the parasite. Furthermore, sera were pre-incubated on *A. lumbricoides*-coated plates and then transferred to *T. canis*-coated plates. By applying this method, we limited the possibility that *Toxocara* spp. positivity is a marker for *Ascaris* spp. exposure. There is little data on cross-reactivity between *Toxocara* and environmental allergens. In our study, no association was observed for *T. canis* IgG4 and allergic sensitization towards HDM. In our study, the RHINESSA offspring were tested for SPT towards cockroach allergen. However, only 3% were sensitized, too few to test for associations with *Ascaris* and *Toxocara* IgG4 positivity.

Our study populations are thoroughly characterized, but the relatively low number of study participants has limited our ability to detect associations. This might be 1 reason that indicated protective associations between helminth exposure and allergic outcomes in the parent population did not reach statistical significance, despite the fact that this population had higher prevalence of *Ascaris* and *Toxocara* seropositivity. We reported association with *Ascaris* spp. seropositivity and growing up on farms with livestock. Given the nature of the exposure and the known association between *Ascaris* spp. and farm animals, especially pigs, it would have been valuable to know which type of livestock the participants had been in contact with. Unfortunately, this information was not captured in the interview or questionnaires. The study design limits our possibility to infer any causal relationship between exposure to *Ascaris* spp. and *Toxocara* spp. However, the associations reported in the present study raise some intriguing possibilities of zoonotic helminth exposures as a potential modifier for allergic disease development which warrants more research.

In conclusion, this study shows that in an affluent northern European population, helminth exposure is still relatively common and that *Toxocara* seropositivity appeared to be a strong risk factor for allergic outcomes in the younger generation. *Toxocara* was associated with childhood cat keeping, but this did not explain the associations between *Toxocara* and allergies. The risk associations differed between the parent and offspring generations, and parental *Toxocara* seropositivity was associated with allergic outcomes in their offspring but not in themselves. These intriguing findings might shed light on the increase in allergies during the last decades. Our findings suggest that zoonotic helminth exposure may modify the risk of allergic disease.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTION

NOJ, CS, WGCH and RJB contributed to conception and design of the study and drafted the manuscript. NOJ, CS, AN, SPS, FGR, WGCH and RJB were involved in acquisition of data. NOJ, CS, JI, WGCH and RJB were involved in statistical analysis. All authors contributed to interpretation of data for the work, revised the manuscript critically for important intellectual content and approved the final version of the manuscript.

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REFERENCES

1. von Mutius E. The microbial environment and its influence on asthma prevention in early life. *J Allergy Clin Immunol*. 2016;137:680-689.
2. Stein MM, Hrusch CL, Gozdz J, et al. Innate immunity and asthma risk in Amish and Hutterite farm children. *N Engl J Med*. 2016;375:411-421.
3. Sigurs N, Aljassim F, Kjellman B, et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. *Thorax*. 2010;65:1045-1052.
4. Maizels RM, McSorley HJ. Regulation of the host immune system by helminth parasites. *J Allergy Clin Immunol*. 2016;138:666-675.
5. Grainger JR, Smith KA, Hewitson JP, et al. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-beta pathway. *J Exp Med*. 2010;207:2331-2341.
6. Hussaarts L, van der Vlugt LE, Yazdanbakhsh M, Smits HH. Regulatory B-cell induction by helminths: implications for allergic disease. *J Allergy Clin Immunol*. 2011;128:733-739.
7. McSorley HJ, Blair NF, Smith KA, McKenzie AN, Maizels RM. Blockade of IL-33 release and suppression of type 2 innate lymphoid cell responses by helminth secreted products in airway allergy. *Mucosal Immunol*. 2014;7:1068-1078.
8. Heylen M, Ruyssers NE, Gielis EM, et al. Of worms, mice and man: an overview of experimental and clinical helminth-based therapy for inflammatory bowel disease. *Pharmacol Ther*. 2014;143:153-167.
9. Mpairwe H, Ndibazza J, Webb EL, et al. Maternal hookworm modifies risk factors for childhood eczema: results from a birth cohort in Uganda. *Pediatr Allergy Immunol*. 2014;25:481-488.

10. Mpairwe H, Webb EL, Muhangi L, et al. Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. *Pediatr Allergy Immunol*. 2011;22:305-312.

11. van den Biggelaar AH, Rodrigues LC, van Ree R, et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. *J Infect Dis*. 2004;189:892-900.

12. Smits HH, Hiemstra PS, Prazeres da Costa C, et al. Microbes and asthma: Opportunities for intervention. *J Allergy Clin Immunol*. 2016;137:690-697.

13. Strunz EC, Addiss DG, Stocks ME, Ogden S, Utzinger J, Freeman MC. Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis. *PLoS Med*. 2014;11:e1001620.

14. Mughini-Gras L, Harms M, van Pelt W, Pinelli E, Kortbeek T. Seroepidemiology of human Toxocara and Ascaris infections in the Netherlands. *Parasitol Res*. 2016;115:3779-3794.

15. Pinelli E, Herremans T, Harms MG, Hoek D, Kortbeek LM. Toxocara and Ascaris seropositivity among patients suspected of visceral and ocular larva migrans in the Netherlands: trends from 1998 to 2009. *Eur J Clin Microbiol Infect Dis*. 2011;30:873-879.

16. Lassen B, Janson M, Viltrop A, et al. Serological Evidence of Exposure to Globally Relevant Zoonotic Parasites in the Estonian Population. *PLoS One*. 2016;11:e0164142.

17. Bendall RP, Barlow M, Betson M, Stothard JR, Nejsum P. Zoonotic ascariasis, United Kingdom. *Emerg Infect Dis*. 2011;17:1964-1966.

18. Gipson K, Avery R, Shah H, et al. Loffler syndrome on a Louisiana pig farm. *Respiratory Med Case Rep*. 2016;19:128-131.

19. Miller LA, Colby K, Manning SE, et al. Ascariasis in humans and pigs on small-scale farms, Maine, USA, 2010-2013. *Emerg Infect Dis*. 2015;21:332-334.

20. Nejsum P, Parker ED Jr, Frydenberg J, et al. Ascariasis is a zoonosis in denmark. *J Clin Microbiol*. 2005;43:1142-1148.

21. Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clin Microbiol Rev*. 2003;16:265-272.

22. Cassenote AJ, Lima AR, Pinto Neto JM, Rubinsky-Elefant G. Seroprevalence and modifiable risk factors for Toxocara spp. in Brazilian schoolchildren. *PLoS Negl Trop Dis*. 2014;8:e2830.

23. Fernando D, Wickramasinghe P, Kapilananda G, Dewasurendra RL, Amarasooriya M, Dayaratne A. Toxocara seropositivity in Sri Lankan children with asthma. *Pediatr Int*. 2009;51:241-245.

24. Fu CJ, Chuang TW, Lin HS, et al. Seroepidemiology of Toxocara canis infection among primary schoolchildren in the capital area of the Republic of the Marshall Islands. *BMC Infect Dis*. 2014;14:261.

25. Mendonca LR, Veiga RV, Dattoli VC, et al. Toxocara seropositivity, atopy and wheezing in children living in poor neighbourhoods in urban Latin American. *PLoS Negl Trop Dis*. 2012;6:e1886.

26. Sharghi N, Schantz PM, Caramico L, Ballas K, Teague BA, Hotez PJ. Environmental exposure to Toxocara as a possible risk factor for asthma: a clinic-based case-control study. *Clin Infect Dis*. 2001;32: E111-E116.

27. Dogan N, Dinleyici EC, Bor O, Toz SO, Ozbel Y. Seroepidemiological survey for Toxocara canis infection in the northwestern part of Turkey. *Turkiye Parazitol Derg*. 2007;31:288-291.

28. Won KY, Kruszon-Moran D, Schantz PM, Jones JL. National seroprevalence and risk factors for Zoonotic Toxocara spp. infection. *Am J Trop Med Hyg*. 2008;79:552-557.

29. Gonzalez-Quintela A, Gude F, Campos J, et al. Toxocara infection seroprevalence and its relationship with atopic features in a general adult population. *Int Arch Allergy Immunol*. 2006;139:317-324.

30. Chan PW, Anuar AK, Fong MY, Debruyne JA, Ibrahim J. Toxocara seroprevalence and childhood asthma among Malaysian children. *Pediatr Int*. 2001;43:350-353.

31. Kuk S, Ozel E, Oguzturk H, Kirkil G, Kaplan M. Seroprevalence of Toxocara antibodies in patients with adult asthma. *South Med J*. 2006;99:719-722.

32. Buijs J, Borsboom G, Renting M, et al. Relationship between allergic manifestations and Toxocara seropositivity: a cross-sectional study among elementary school children. *Eur Respir J*. 1997;10:1467-1475.

33. Buijs J, Borsboom G, van Gemund JJ, et al. Toxocara seroprevalence in 5-year-old elementary schoolchildren: relation with allergic asthma. *Am J Epidemiol*. 1994;140:839-847.

34. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J*. 1994;7:954-960.

35. Bobat S, Darby M, Mrdjen D, et al. Natural and vaccine-mediated immunity to Salmonella Typhimurium is impaired by the helminth Nippostrongylus brasiliensis. *PLoS Negl Trop Dis*. 2014;8:e3341.

36. Kennedy MW, Qureshi F, Fraser EM, Haswell-Elkins MR, Elkins DB, Smith HV. Antigenic relationships between the surface-exposed, secreted and somatic materials of the nematode parasites Ascaris lumbricoides, Ascaris suum, and Toxocara canis. *Clin Exp Immunol*. 1989;75:493-500.

37. Nieuwenhuizen NE, Lopata AL. Anisakis—a food-borne parasite that triggers allergic host defences. *Int J Parasitol*. 2013;43:1047-1057.

38. Ma G, Holland CV, Wang T, et al. Human toxocariasis. *Lancet Infect Dis*. 2017. doi: 10.1016/S1473-3099(17)30331-6. [Epub ahead of print]

39. Maizels RM. Toxocara canis: molecular basis of immune recognition and evasion. *Vet Parasitol*. 2013;193:365-374.

40. Avila LF, Telmo Pde L, Martins LH, et al. Protective effect of the probiotic Saccharomyces boulardii in Toxocara canis infection is not due to direct action on the larvae. *Rev Inst Med Trop Sao Paulo*. 2013;55:363-365.

41. Moreira GM, Telmo Pde L, Mendonca M, et al. Human toxocariasis: current advances in diagnostics, treatment, and interventions. *Trends Parasitol*. 2014;30:456-464.

42. Elliott AM, Mpairwe H, Quigley MA, et al. Helminth infection during pregnancy and development of infantile eczema. *JAMA*. 2005;294:2032-2034.

43. Harris NL, Spoerri I, Schopfer JF, et al. Mechanisms of neonatal mucosal antibody protection. *J Immunol*. 2006;177:6256-6262.

44. Bertelsen RJ, Rava M, Carsin AE, et al. Clinical markers of asthma and IgE assessed in parents before conception predict asthma and hayfever in the offspring. *Clin Exp Allergy*. 2017;47:627-638.

45. Soubry A, Hoyo C, Jirtle RL, Murphy SK. A paternal environmental legacy: evidence for epigenetic inheritance through the male germ line. *BioEssays*. 2014;36:359-371.

46. Arshad SH, Karmaus W, Raza A, et al. The effect of parental allergy on childhood allergic diseases depends on the sex of the child. *J Allergy Clin Immunol*. 2012;130:e6.

47. Manolio TA, Barnes KC, Beaty TH, Levett PN, Naidu RP, Wilson AF. Sex differences in heritability of sensitization to Blomia tropicalis in asthma using regression of offspring on midparent (ROMP) methods. *Hum Genet*. 2003;113:437-446.

48. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol*. 2014;5: 520.

49. Beaver PC. The nature of visceral larva migrans. *J Parasitol*. 1969;55:3-12.

50. Kidd CD, Thompson PJ, Barrett L, Baltic S. Histone Modifications and Asthma. The Interface of the Epigenetic and Genetic Landscapes. *Am J Respir Cell Mol Biol*. 2016;54:3-12.

51. Kahr N, Naeser V, Stensballe LG, et al. Gene-environment interaction in atopic diseases: a population-based twin study of early-life exposures. *Clin Respir J*. 2015;9:79-86.

52. Kinder JM, Stelzer IA, Arck PC, Way SS. Immunological implications of pregnancy-induced microchimerism. *Nat Rev Immunol*. 2017;17:483-494.

53. Fedulov AV, Kobzik L. Allergy risk is mediated by dendritic cells with congenital epigenetic changes. *Am J Respir Cell Mol Biol*. 2011;44: 285-292.

54. Matson AP, Zhu L, Lingenheld EG, et al. Maternal transmission of resistance to development of allergic airway disease. *J Immunol*. 2007;179:1282-1291.

55. Fitzsimmons CM, Falcone FH, Dunne DW. Helminth Allergens, Parasite-Specific IgE, and Its Protective Role in Human Immunity. *Front Immunol*. 2014;5:61.

56. Nakazawa T, Khan AF, Yasueda H, et al. Immunization of rabbits with nematode Ascaris lumbricoides antigens induces antibodies cross-reactive to house dust mite Dermatophagoides farinae antigens. *Biosci Biotechnol Biochem*. 2013;77:145-150.

57. Jin Y, Shen C, Huh S, Sohn WM, Choi MH, Hong ST. Serodiagnosis of toxocariasis by ELISA using crude antigen of Toxocara canis larvae. *Korean J Parasitol*. 2013;51:433-439.

58. Nieuwenhuizen NE, Meter JM, Horsnell WG, et al. A cross-reactive monoclonal antibody to nematode haemoglobin enhances protective immune responses to Nippostrongylus brasiliensis. *PLoS Negl Trop Dis*. 2013;7:e2395.

59. Noordin R, Smith HV, Mohamad S, Maizels RM, Fong MY. Comparison of IgG-ELISA and IgG4-ELISA for Toxocara serodiagnosis. *Acta Trop*. 2005;93:57-62.

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