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

*Nils Oskar Jõgi[a] MD and *Cecilie Svanes[b,c] MD, PhD; Silver Peeter Siiak[a] MD; Erin Logan[d]; John W. Holloway[e] PhD; Jannicke Igland[f] PhD; Ane Johannessen[b] PhD; Michael Levin[d] MD, PhD; Francisco Gomez Real[g] MD, PhD; Vivi Schlunssen[h,i] MD, PhD;**William GC Horsnell[j,k,l] PhD and **Randi J. Bertelsen[c,g]PhD.

*Contributed equally

**Contributed equally

Affiliations:

[a]University of Tartu, Tartu, Estonia; [b]Centre for International Health, University of Bergen, Bergen Norway; [c]Department of Occupational Medicine, Haukeland University Hospital, Bergen, Norway; [d]University of Cape Town, Cape Town, South Africa; [e]Human Development & Health, Faculty of Medicine, University of Southampton, Southampton, UK; [f]Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway; [g]Department of Clinical Science, University of Bergen, Bergen, Norway; [h]Aarhus University, Aarhus , Denmark; [i]National Research Centre for the Working Environment, Copenhagen, Denmark; [j]Institute of Infectious Disease and Molecular Medicine/Division of Immunology, University of Cape Town, Cape Town, South Africa; [k]Institute of Microbiology and Infection, University of Birmingham, B15 2TT Birmingham, UK, [l]Laboratory of Molecular and Experimental Immunology and Neurogenetics, UMR 7355, CNRS-University of Orleans and Le Studium Institute for Advanced Studies, Rue Dupanloup, 45000 Orléans, France

Corresponding author: Randi J. Bertelsen, University of Bergen, Department of Clinical Science, P.O. Box 7804, N-5020 Bergen, Norway. Phone: +4755975553. Fax: + 55975890 e-mail: randi.j.bertelsen@uib.no

ABSTRACT

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 sensitisation, in two generations in Bergen, Norway.

Methods: Serum levels of total IgG4, anti-*Toxocara* spp. IgG4 and *Ascaris* spp. IgG4 were established by ELISA in two 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]), hay fever (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.

Word-count: 262/300

Capsule summary

Toxocara appears to be a hitherto unknown risk factor for allergies in Norway, present in 8% of a younger population. Zoonotic helminth exposure should be considered when assessing risks for allergies in affluent countries.

Abbreviations used:

BMI: body mass index

ECRHS: European Community Respiratory Health Survey

ELISA: enzyme-linked immunosorbent assay

IgE: immunoglobulin E

IgG: immunoglobulin G

PBS: phosphate-buffered saline

PBS-BSA: PBS containing 1% bovine serum albumin

RHINESSA: Respiratory Health In Northern Europe, Spain and Australia

RSV: respiratory syncytial virus

spp.: species

SPT: skin prick test

INTRODUCTION

Exposure to microorganisms may alter subsequent risk for 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 type2/TH2 immune response. This is characterised by helminth interaction with epithelial cells causing epithelial release of alarmin cytokines (e.g. IL-25/IL-33) which drive innate lymphoid type 2 (ILC2) secretion of IL-4, IL-5 and IL-13. The interleukins support the induction of characteristic eosinophilia and M2 macrophage polarisation along with CD4 T cell polarisation 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 products that directly influence host immunity by, for example, raising regulatory immune response components (e.g. 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 finding, for example studies have shown exposure to helminths results in raised TReg associated responses and reduced risk of allergy in children [8]. Presence of helminth infection may be required for this protection as anti-helminth therapy can lead to increased prevalence of allergic disease in helminth endemic regions [9]. 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 [10].

However, helminth exposure may not always be protective against allergy. In high-income countries, the major human parasitic helminths that associated with allergy protection have been controlled by strong public health provision breaking the cycle of infection [12, 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. [23]. Levels of human exposure to these parasites can vary from 2-62% 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 [22, 23, 29-31]. However, a proportion of infections can provoke pathologies with rheumatic, neurologic and asthmatic symptoms [24, 32, 33]. Zoonotic *Toxocara* spp. exposure is also associated with increased risk of asthma and atopy [24, 25, 30, 34, 35].

In the present study, we aimed to address how common seroprevalence to *Ascaris* spp. and *Toxocara* spp. was in a Norwegian transgenerational cohort. Seropositivity to both parasites has been reported in Northern Europe. As outlined above *Ascaris* spp. seropositivity can reflect a number of scenarios; such as exposure to *A. lumbricoides*, exposure to the closely related *A. suum* or non-specific binding. Exposure to *Toxocara* spp. is a global feature of exposure to companion animals. 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.

METHODS

Study population

This study is based on information and samples from Norwegian participants in two linked studies, the European Community Respiratory Health Survey (ECRHS; www.ecrhs.org) [36] 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 general populations aged 20-44 years in 1992-1994) that were followed up in 2010-13. 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 E-s (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.

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.

Allergic sensitisation and diseases

Allergic sensitization was determined by skin prick tests (SPT) to 12 allergens (ALK-Abello): Timothy grass, ragweed*, Dermatophagoides pteronyssinus, Dermatophagoides farinea*, cat, dog, birch, *Blatella germanica*, olive, *Alternaria* spp*., Cladosporium* spp*., and Parietaria* spp., 0.9% saline and 10mg/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 wheal size was 3mm greater than the negative control. Blood samples were collected and sera separated in SST vacutainer glasses, centrifuged within 30-60 minutes after collection (at 3400 rpm for 10min, room temperature). The samples were stored at -20˚ C. Total IgE and specific IgE were performed according to standardised laboratory methods in Haukeland University Hospital in Bergen, Norway. IgE positivity was defined by IgE≥0.35 kU/L to at least one of four allergens tested (cat, timothy grass, and house dust mite).

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

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 four times with distilled water. Worms were then homogenized in filter sterilized phosphate-buffered saline (PBS). The homogenate was centrifuged at 12000 rpm for 20 mins and the soluble fraction collected and filtered through a 0.20 µm filter. Protein concentration of soluble worm antigen preparations was established using a bivinvoninic acid protein assay by Thermo Scientific (Rockford, IL, USA).

Detection of total IgG4, Toxocara canis and Ascaris lumbricoides specific antibodies by ELISA

Analysis of IgG4 towards *T. canis* and *A. lumbricoides* were performed for sera from both ECRHS3 and RHINESSA Bergen participants, whereas total IgG4 were 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 (Sigma-Aldrich). 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. 96-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 verses sample dilution curve.[37]

Anti-helminth immunoglobulin responses can be cross-reactive between helminth species.[38, 39] 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.

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

189 categorized as primary school, secondary/technical education, and college/university, whereas the
190 adolescents were categorized as being students/still in school.

191

192 **Statistical analyses**

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

195 Logistic regression was applied to assess associations between pet keeping, place of upbringing, age
196 and sex as well as other potential variables that might be associated with helminth exposure (anti-
197 *Toxocara* and anti-*Ascaris* IgG4 positivity). Similar models were applied to study associations
198 between *Toxocara* seropostitivity and allergic sensitization (specific IgE and SPT towards inhalant
199 allergens), total IgE, rhinitis, hay fever and asthma. Models were performed separately for the two
200 generations (ECRHS parent and RHINESSA offspring generations). In all regression models with the
201 combined study populations, we corrected for clustering within families (parent-offspring and
202 siblings) by applying a cluster for family-id. To discriminate between helminth infections that can
203 translate into high specific IgE which does not necessarily reflect SPT reactivity, we performed
204 sensitivity analyses with separate models for *Toxocara* and *Ascaris* IgG4 positivity and associations
205 with SPT and specific IgE towards any inhalant, cat, HDM and Timothy (grass) allergens.

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

207

RESULTS

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 a cat in childhood, but there was no statistical difference in dog keeping between the two generations (Table 1). Current pet keeping was similar between the two cohorts. Of the parent generation 15% had grown up on a farm with livestock, compared to only 0.8% of the offspring generation. The two generations had similar life-time prevalence of rhinitis and hay fever, but the offspring generation had a higher prevalence of positive allergy test (positive SPT or IgE towards at least one inhalant allergens) than the parent generation (44.5% vs 31.1%, respectively, p=0.006) (Table 1).

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=0.002 (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 were 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 combinded (p=0.07, 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.

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 (Tables 2a and 2b). *Toxocara* spp. seropositivity was associated with pet keeping before the age of 15 among the offspring (OR=6.15[1.37-27.54], p=0.02), 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=0.04 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).

Exposure to helminths as associated with risk of allergic sensitisation and diseases

In the offspring generation, anti-*Toxocara* IgG4 was associated with three to four times increased risk of reported wheeze, hay fever 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=0.34). 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 specific IgE and/or SPT positive results combined outcomes for offspring *Toxocara* spp. positivity and timothy grass sensitisation, with positive association for 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 allergen (aOR=2.67 (0.99, 7.24), p=0.05), whereas the association was non-significant for *Ascaris* spp. and specific IgE towards cat allergens (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).

DISCUSSION

In this study, we present evidence that exposure to helminths exists in a Norwegian population and that exposure to these parasites 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 found a higher prevalence of exposure (defined by ELISA detectable anti-*Toxocara* spp. and anti-*Ascaris* spp. IgG4s) in participants born between 1945 and 1972 (parents) compared to participants born between 1969 and 2003 (offspring). Detection of elevated levels of total IgG4 was primarily related to elevated levels of anti-*Toxocara* spp. IgG4. 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. In the offspring generation, *Toxocara* spp. seropositivity was associated with increased risk of allergic sensitization and diseases, with 3-4 times increased risk for 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 US, with 8-11% reported for Dutch children [34, 35], 8% for Dutch adults [14], 14% in a US population-based study (NHANES) [31], but lower than among Spanish adults (29%) [30]. We found that *Toxocara* spp. seropositivity was associated with allergic diseases and sensitisation among the offspring. This is in agreement with findings reported from other regions; for example Mughini-Gras et al. reported an association of anti-*Toxocara* IgGs with hay fever in the Netherlands [14]. 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-asthmatics) [32], Turkey (13% of asthmatics and 2% of non-asthmatics) [33], and Sri-Lanka (29% of asthmatics

297 and 10% in non-asthmatics) [24]. Why zoonotic *Toxocara* spp. infections do not confer the protection
298 associated with natural helminth infections is not known, but may possibly be due to *Toxocara* spp.
299 secreted immune regulatory products being active against dog and cat immune responses and not
300 against human [41]. Therefore, the *Toxocara* spp. induced type 2 immune response may lack
301 effective regulation in a human host promoting allergic pathology and potentially acting as a risk
302 factor for sensitization to other allergens [42].

303

304 An unexpected finding from our study was the large differences in allergic risk-association between
305 the parent and offspring generations. No effects from *Toxocara* spp. seropositivity were indicated in
306 the parent generation. However, *Toxocara* spp. seropositivity was strongly associated with increased
307 risk for allergic outcomes in the offspring generation. This may imply that early life exposure to
308 *Toxocara* is a risk factor for development of allergic disease and that the risk resulting from this
309 exposure may not be life-long. Changes in microbial diversity in the environment might potentially be
310 of importance for response to helminths, as it has been demonstrated that gut microbiota can alter
311 the response to *Toxocara* in mice [43].

312

313 The age effect in our study, with increasing *Toxocara* seroprevalence with age in the offspring
314 generation, might reflect a time trend rather than a biological age patterns, and decreasing
315 seroprevalence of *Toxocara* in more recent birth cohorts is presented in Figure 1. Similar trends were
316 observed for *Ascaris*. Comparison of offspring and parents in the present study indicated that both
317 *Toxocara* and *Ascaris* exposure were lower in offspring. Rural areas tend to exhibit higher prevalence
318 of human exposure to helminths (35–42%) than semi-rural (15–20%) or urban (2–5%) areas [44].
319 Although urban living is the most fitting description of the populations in the present study, urban
320 areas, such as parks and town squares, can contain high numbers of *Toxocara* eggs and may
321 represent a substantial risk factor for infection by *Toxocara* spp. [8, 45, 46]. Indeed, some studies

have reported higher levels of *Toxocara* seroprevalence among subjects living in urban compared to rural areas [29, 30].

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 [51], and there are mice models demonstrating that maternal helminth infection influences immunological characteristics in pups [52]. With regard to differential risk-outcome by gender, research focused on other exposures than helminths, have found that parental pre-conception exposure might influence disease risk in offspring differently through the maternal and paternal lines [47, 48]. The risk of allergy has been reported to not only be affected by the maternal or paternal line, but also to depend on the sex of the child [47, 49, 50]. 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 [53], but *Toxocara* larvae may stay capsulated in tissues for many years in accidental hosts [54]. Our findings may suggest either ongoing antigenic exposure or the existence of long lived memory/plasma B cell populations as 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 [55], shared environment [56], microchimerism [57] or passive transfer of immune markers or antibodies [58, 59].

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 recognised zoonosis 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 be reflective of non-specific ELISA data 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 [60], and cross reactivity between *Ascaris lumbricoides* and *Dermatophagoides farinae* IgG has been demonstrated in rabbits [61]. However, as there are only a small number of documented cases of toxocariasis ever described in Norway, we would not expect our study participants to have specific IgE towards *Toxocara* spp. we therefore chose to only assess IgG4 towards *Toxocara* spp., which is the most common used serology tool, even when it comes to serodiagnosis of toxocariasis [62]. 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 recognised as being cross-reactivity to other helminth antigens [63]; in particular species within the group of ascaridoid nematodes, such as *A. lumbricoides*, *A. suum*, and *T. canis,* show antigenic relationship [38]. Thus, detection of anti-*Ascaris* IgG4 may be reflective of 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.[62]. Moreover, we based our definition of exposure on elevated levels of *Toxocara* spp. IgG4

which lends greater specificity [64] 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 limit 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, and, thus, too few to test for associations with *Ascaris* and *Toxocara* IgG4 positivity.

The study populations are thoroughly characterised, but the relatively low number of study participants may have limited our ability to detect associations. This might be one 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 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 modified 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 for allergic disease..

406

407 **ACKNOWLEDGEMENTS**

408 This project was funded by a World Universities Network (WUN) Research Development Fund award
409 for the project "Helminths and allergy in South-Africa and Northern Europe". RJ Bertelsen is
410 supported by the Research Council of Norway, grant number 230827. The Bergen RHINESSA study is
411 funded by the Research Council of Norway (Grants No. 214123 and 228174), the Bergen Medical
412 Research Foundation, the Western Norwegian Regional Health Authorities (Grants No. 912011,
413 911892 and 911631), the Norwegian Labour Inspection and the Norwegian Asthma and Allergy
414 Association. The project has received funding from the European Union's Horizon 2020 research and
415 innovation programme under grant agreement No 633212.

416 We have no conflict of interest to declare.

417

418 **Author contributions**

419 NOJ, CS, WGCH and RJB contributed with conception and design of the study and drafting of the
420 manuscript, NOJ, CS, AN, SPS, FGR, WGCH and RJB with acquisition of data, NOJ, CS, JI, WGCH and
421 RJB with statistical analysis, and all authors contributed with interpretation of data for the work and
422 revising the manuscript critically for important intellectual content. All authors approved the final
423 version of the manuscript.

424

425

426

427

428

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, Igartua C, Pivniouk V, Murray SE, Ledford JG, Marques dos Santos M, Anderson RL, Metwali N, Neilson JW, Maier RM, Gilbert JA, Holbreich M, Thorne PS, Martinez FD, von Mutius E, Vercelli D, Ober C, Sperling AI. Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children. The New England journal of medicine 2016; 375:411-421.
3. Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarnason R, Gustafsson PM. 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, McSorley HJ, Harcus Y, Filbey KJ, Finney CA, Greenwood EJ, Knox DP, Wilson MS, Belkaid Y, Rudensky AY, Maizels RM. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-beta pathway. The Journal of experimental medicine 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 immunology 2014; 7:1068-1078.
8. Cooper PJ, Chico M, Sandoval C, Espinel I, Guevara A, Levine MM, Griffin GE, Nutman TB. Human infection with Ascaris lumbricoides is associated with suppression of the interleukin-2 response to recombinant cholera toxin B subunit following vaccination with the live oral cholera vaccine CVD 103-HgR. Infection and immunity 2001; 69:1574-1580.
9. van den Biggelaar AH, Rodrigues LC, van Ree R, van der Zee JS, Hoeksma-Kruize YC, Souverijn JH, Missinou MA, Borrmann S, Kremsner PG, Yazdanbakhsh M. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 2004; 189:892-900.
10. Smits HH, Hiemstra PS, Prazeres da Costa C, Ege M, Edwards M, Garn H, Howarth PH, Jartti T, de Jong EC, Maizels RM, Marsland BJ, McSorley HJ, Muller A, Pfefferle PI, Savelkoul H, Schwarze J, Unger WW, von Mutius E, Yazdanbakhsh M, Taube C. Microbes and asthma: Opportunities for intervention. J Allergy Clin Immunol 2016; 137:690-697.
11. Murray CJ, Ortblad KF, Guinovart C, Lim SS, Wolock TM, Roberts DA, Dansereau EA, Graetz N, Barber RM, Brown JC, Wang H, Duber HC, Naghavi M, Dicker D, Dandona L, Salomon JA, Heuton KR, Foreman K, Phillips DE, Fleming TD, Flaxman AD, Phillips BK, Johnson EK, Coggeshall MS, Abd-Allah F, Abera SF, Abraham JP, Abubakar I, Abu-Raddad LJ, Abu-Rmeileh NM, Achoki T, Adeyemo AO, Adou AK, Adsuar JC, Agardh EE, Akena D, Al Kahbouri MJ, Alasfoor D, Albittar MI, Alcala-Cerra G, Alegretti MA, Alemu ZA, Alfonso-Cristancho R, Alhabib S, Ali R, Alla F, Allen PJ, Alsharif U, Alvarez E, Alvis-Guzman N, Amankwaa AA, Amare AT, Amini H, Ammar W, Anderson BO, Antonio CA, Anwari P, Arnlov J, Arsenijevic VS, Artaman A, Asghar RJ, Assadi R, Atkins LS, Badawi A, Balakrishnan K, Banerjee A, Basu S, Beardsley J, Bekele T, Bell ML, Bernabe E, Beyene TJ, Bhala N, Bhalla A, Bhutta ZA, Abdulhak AB, Binagwaho A, Blore JD, Basara BB, Bose D, Brainin M, Breitborde N, Castaneda-Orjuela CA, Catala-Lopez F, Chadha VK, Chang JC, Chiang PP, Chuang TW, Colomar M, Cooper LT, Cooper C, Courville KJ, Cowie BC, Criqui MH, Dandona R, Dayama A, De Leo D, Degenhardt L, Del Pozo-Cruz B, Deribe K, Des Jarlais DC, Dessalegn M, Dharmaratne SD, Dilmen U, Ding EL, Driscoll TR, Durrani AM, Ellenbogen RG, Ermakov SP, Esteghamati A, Faraon EJ, Farzadfar F, Fereshtehnejad SM, Fijabi DO, Forouzanfar MH, Fra Paleo U, Gaffikin L, Gamkrelidze A, Gankpe FG, Geleijnse JM, Gessner BD, Gibney KB, Ginawi IA, Glaser EL, Gona P, Goto A, Gouda HN, Gugnani HC, Gupta R, Gupta R, Hafezi-Nejad N, Hamadeh RR, Hammami M, Hankey GJ, Harb HL, Haro JM, Havmoeller R, Hay SI, Hedayati

MT, Pi IB, Hoek HW, Hornberger JC, Hosgood HD, Hotez PJ, Hoy DG, Huang JJ, Iburg KM, Idrisov BT, Innos K, Jacobsen KH, Jeemon P, Jensen PN, Jha V, Jiang G, Jonas JB, Juel K, Kan H, Kankindi I, Karam NE, Karch A, Karema CK, Kaul A, Kawakami N, Kazi DS, Kemp AH, Kengne AP, Keren A, Kereselidze M, Khader YS, Khalifa SE, Khan EA, Khang YH, Khonelidze I, Kinfu Y, Kinge JM, Knibbs L, Kokubo Y, Kosen S, Defo BK, Kulkarni VS, Kulkarni C, Kumar K, Kumar RB, Kumar GA, Kwan GF, Lai T, Balaji AL, Lam H, Lan Q, Lansingh VC, Larson HJ, Larsson A, Lee JT, Leigh J, Leinsalu M, Leung R, Li Y, Li Y, De Lima GM, Lin HH, Lipshultz SE, Liu S, Liu Y, Lloyd BK, Lotufo PA, Machado VM, Maclachlan JH, Magis-Rodriguez C, Majdan M, Mapoma CC, Marcenes W, Marzan MB, Masci JR, Mashal MT, Mason-Jones AJ, Mayosi BM, Mazorodze TT, McKay AC, Meaney PA, Mehndiratta MM, Mejia-Rodriguez F, Melaku YA, Memish ZA, Mendoza W, Miller TR, Mills EJ, Mohammad KA, Mokdad AH, Mola GL, Monasta L, Montico M, Moore AR, Mori R, Moturi WN, Mukaigawara M, Murthy KS, Naheed A, Naidoo KS, Naldi L, Nangia V, Narayan KM, Nash D, Nejjari C, Nelson RG, Neupane SP, Newton CR, Ng M, Nisar MI, Nolte S, Norheim OF, Nowaseb V, Nyakarahuka L, Oh IH, Ohkubo T, Olusanya BO, Omer SB, Opio JN, Orisakwe OE, Pandian JD, Papachristou C, Caicedo AJ, Patten SB, Paul VK, Pavlin BI, Pearce N, Pereira DM, Pervaiz A, Pesudovs K, Petzold M, Pourmalek F, Qato D, Quezada AD, Quistberg DA, Rafay A, Rahimi K, Rahimi-Movaghar V, Ur Rahman S, Raju M, Rana SM, Razavi H, Reilly RQ, Remuzzi G, Richardus JH, Ronfani L, Roy N, Sabin N, Saeedi MY, Sahraian MA, Samonte GM, Sawhney M, Schneider IJ, Schwebel DC, Seedat S, Sepanlou SG, Servan-Mori EE, Sheikhbahaei S, Shibuya K, Shin HH, Shiue I, Shivakoti R, Sigfusdottir ID, Silberberg DH, Silva AP, Simard EP, Singh JA, Skirbekk V, Sliwa K, Soneji S, Soshnikov SS, Sreeramareddy CT, Stathopoulou VK, Stroumpoulis K, Swaminathan S, Sykes BL, Tabb KM, Talongwa RT, Tenkorang EY, Terkawi AS, Thomson AJ, Thorne-Lyman AL, Towbin JA, Traebert J, Tran BX, Dimbuene ZT, Tsilimbaris M, Uchendu US, Ukwaja KN, Uzun SB, Vallely AJ, Vasankari TJ, Venketasubramanian N, Violante FS, Vlassov VV, Vollset SE, Waller S, Wallin MT, Wang L, Wang X, Wang Y, Weichenthal S, Weiderpass E, Weintraub RG, Westerman R, White RA, Wilkinson JD, Williams TN, Woldeyohannes SM, Wong JQ, Xu G, Yang YC, Yano Y, Yentur GK, Yip P, Yonemoto N, Yoon SJ, Younis M, Yu C, Jin KY, El Sayed Zaki M, Zhao Y, Zheng Y, Zhou M, Zhu J, Zou XN, Lopez AD, Vos T. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014; 384:1005-1070.

12. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet 2006; 368:1106-1118.

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 medicine 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.

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, Neare K, Hutt P, Golovljova I, Tummeleht L, Jokelainen P. 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. Emerging infectious diseases 2011; 17:1964-1966.

18. Gipson K, Avery R, Shah H, Pepiak D, Begue RE, Malone J, Wall LA. Loffler syndrome on a Louisiana pig farm. Respiratory medicine case reports 2016; 19:128-131.

19. Miller LA, Colby K, Manning SE, Hoenig D, McEvoy E, Montgomery S, Mathison B, de Almeida M, Bishop H, Dasilva A, Sears S. Ascariasis in humans and pigs on small-scale farms, Maine, USA, 2010-2013. Emerging infectious diseases 2015; 21:332-334.

20. Nejsum P, Parker ED, Jr., Frydenberg J, Roepstorff A, Boes J, Haque R, Astrup I, Prag J, Skov Sorensen UB. Ascariasis is a zoonosis in denmark. Journal of clinical microbiology 2005; 43:1142-1148.

21. Archelli S, Santillan GI, Fonrouge R, Cespedes G, Burgos L, Radman N. Toxocariasis: seroprevalence in abandoned-institutionalized children and infants. Rev Argent Microbiol 2014; 46:3-6.
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. Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev 2003; 16:265-272.
24. Fernando D, Wickramasinghe P, Kapilananda G, Dewasurendra RL, Amarasooriya M, Dayaratne A. Toxocara seropositivity in Sri Lankan children with asthma. Pediatrics international : official journal of the Japan Pediatric Society 2009; 51:241-245.
25. Mendonca LR, Veiga RV, Dattoli VC, Figueiredo CA, Fiaccone R, Santos J, Cruz AA, Rodrigues LC, Cooper PJ, Pontes-de-Carvalho LC, Barreto ML, Alcantara-Neves NM. Toxocara seropositivity, atopy and wheezing in children living in poor neighbourhoods in urban Latin American. PLoS Negl Trop Dis 2012; 6:e1886.
26. Paludo ML, Falavigna DL, Elefant GR, Gomes ML, Baggio ML, Amadei LB, Falavigna-Guilherme AL. Frequency of Toxocara infection in children attended by the health public service of Maringa, south Brazil. Rev Inst Med Trop Sao Paulo 2007; 49:343-348.
27. 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:E111-116.
28. Walsh MG. Toxocara infection and diminished lung function in a nationally representative sample from the United States population. Int J Parasitol 2011; 41:243-247.
29. 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.
30. Gonzalez-Quintela A, Gude F, Campos J, Garea MT, Romero PA, Rey J, Meijide LM, Fernandez-Merino MC, Vidal C. Toxocara infection seroprevalence and its relationship with atopic features in a general adult population. International archives of allergy and immunology 2006; 139:317-324.
31. Won KY, Kruszon-Moran D, Schantz PM, Jones JL. National seroprevalence and risk factors for Zoonotic Toxocara spp. infection. The American journal of tropical medicine and hygiene 2008; 79:552-557.
32. Chan PW, Anuar AK, Fong MY, Debruyne JA, Ibrahim J. Toxocara seroprevalence and childhood asthma among Malaysian children. Pediatrics international : official journal of the Japan Pediatric Society 2001; 43:350-353.
33. 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.
34. Buijs J, Borsboom G, Renting M, Hilgersom WJ, van Wieringen JC, Jansen G, Neijens J. Relationship between allergic manifestations and Toxocara seropositivity: a cross-sectional study among elementary school children. The European respiratory journal 1997; 10:1467-1475.
35. Buijs J, Borsboom G, van Gemund JJ, Hazebroek A, van Dongen PA, van Knapen F, Neijens HJ. Toxocara seroprevalence in 5-year-old elementary schoolchildren: relation with allergic asthma. American journal of epidemiology 1994; 140:839-847.
36. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. The European respiratory journal 1994; 7:954-960.
37. Bobat S, Darby M, Mrdjen D, Cook C, Logan E, Auret J, Jones E, Schnoeller C, Flores-Langarica A, Ross EA, Vira A, Lopez-Macias C, Henderson IR, Alexander J, Brombacher F, Horsnell WG, Cunningham AF. Natural and vaccine-mediated immunity to Salmonella Typhimurium is impaired by the helminth Nippostrongylus brasiliensis. PLoS Negl Trop Dis 2014; 8:e3341.
38. 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. Clinical and experimental immunology 1989; 75:493-500.

583 39. Nieuwenhuizen NE, Lopata AL. Anisakis--a food-borne parasite that triggers allergic host
584 defences. Int J Parasitol 2013; 43:1047-1057.
585 40. Mao XQ, Sun DJ, Miyoshi A, Feng Z, Handzel ZT, Hopkin JM, Shirakawa T. The link between
586 helminthic infection and atopy. Parasitol Today 2000; 16:186-188.
587 41. Ma G, Holland CV, Wang T, Hofmann A, Fan CK, Maizels RM, Hotez PJ, Gasser RB. Human
588 toxocariasis. The Lancet Infectious diseases 2017.
589 42. Maizels RM. Toxocara canis: molecular basis of immune recognition and evasion. Vet
590 Parasitol 2013; 193:365-374.
591 43. Avila LF, Telmo Pde L, Martins LH, Glaeser TA, Conceicao FR, Leite FP, Scaini CJ. Protective
592 effect of the probiotic Saccharomyces boulardii in Toxocara canis infection is not due to direct action
593 on the larvae. Rev Inst Med Trop Sao Paulo 2013; 55:363-365.
594 44. Moreira GM, Telmo Pde L, Mendonca M, Moreira AN, McBride AJ, Scaini CJ, Conceicao FR.
595 Human toxocariasis: current advances in diagnostics, treatment, and interventions. Trends Parasitol
596 2014; 30:456-464.
597 45. Maraghi S, Mazhab Jafari K, Sadjjadi SM, Latifi SM, Zibaei M. Study on the contamination of
598 Abadan public parks soil with Toxocara spp. eggs. J Environ Health Sci Eng 2014; 12:86.
599 46. Ruiz de Ybanez MR, Garijo MM, Alonso FD. Prevalence and viability of eggs of Toxocara spp.
600 and Toxascaris leonina in public parks in eastern Spain. J Helminthol 2001; 75:169-173.
601 47. Bertelsen RJ, Rava M, Carsin AE, Accordini S, Benediktsdottir B, Dratva J, Franklin KA, Heinrich
602 J, Holm M, Janson C, Johannessen A, Jarvis DL, Jogi R, Leynaert B, Norback D, Omenaas ER, Raherison
603 C, Sanchez-Ramos JL, Schlunssen V, Sigsgaard T, Dharmage SC, Svanes C. Clinical markers of asthma
604 and IgE assessed in parents before conception predict asthma and hayfever in the offspring. Clinical
605 and experimental allergy : journal of the British Society for Allergy and Clinical Immunology 2017;
606 47:627-638.
607 48. Soubry A, Hoyo C, Jirtle RL, Murphy SK. A paternal environmental legacy: evidence for
608 epigenetic inheritance through the male germ line. BioEssays : news and reviews in molecular,
609 cellular and developmental biology 2014; 36:359-371.
610 49. Arshad SH, Karmaus W, Raza A, Kurukulaaratchy RJ, Matthews SM, Holloway JW,
611 Sadeghnejad A, Zhang H, Roberts G, Ewart SL. The effect of parental allergy on childhood allergic
612 diseases depends on the sex of the child. J Allergy Clin Immunol 2012; 130:427-434 e426.
613 50. Manolio TA, Barnes KC, Beaty TH, Levett PN, Naidu RP, Wilson AF. Sex differences in
614 heritability of sensitization to Blomia tropicalis in asthma using regression of offspring on midparent
615 (ROMP) methods. Human genetics 2003; 113:437-446.
616 51. Elliott AM, Mpairwe H, Quigley MA, Nampijja M, Muhangi L, Oweka-Onyee J, Muwanga M,
617 Ndibazza J, Whitworth JA. Helminth infection during pregnancy and development of infantile
618 eczema. Jama 2005; 294:2032-2034.
619 52. Harris NL, Spoerri I, Schopfer JF, Nembrini C, Merky P, Massacand J, Urban JF, Jr., Lamarre A,
620 Burki K, Odermatt B, Zinkernagel RM, Macpherson AJ. Mechanisms of neonatal mucosal antibody
621 protection. J Immunol 2006; 177:6256-6262.
622 53. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector
623 functions. Frontiers in immunology 2014; 5:520.
624 54. Beaver PC. The nature of visceral larva migrans. The Journal of parasitology 1969; 55:3-12.
625 55. Kidd CD, Thompson PJ, Barrett L, Baltic S. Histone Modifications and Asthma. The Interface of
626 the Epigenetic and Genetic Landscapes. American journal of respiratory cell and molecular biology
627 2016; 54:3-12.
628 56. Kahr N, Naeser V, Stensballe LG, Kyvik KO, Skytthe A, Backer V, Bonnelykke K, Thomsen SF.
629 Gene-environment interaction in atopic diseases: a population-based twin study of early-life
630 exposures. The clinical respiratory journal 2015; 9:79-86.
631 57. Kinder JM, Stelzer IA, Arck PC, Way SS. Immunological implications of pregnancy-induced
632 microchimerism. Nature reviews Immunology 2017; 17:483-494.
633 58. Fedulov AV, Kobzik L. Allergy risk is mediated by dendritic cells with congenital epigenetic
634 changes. American journal of respiratory cell and molecular biology 2011; 44:285-292.

635 59. Matson AP, Zhu L, Lingenheld EG, Schramm CM, Clark RB, Selander DM, Thrall RS, Breen E,
636 Puddington L. Maternal transmission of resistance to development of allergic airway disease. J
637 Immunol 2007; 179:1282-1291.
638 60. Fitzsimmons CM, Falcone FH, Dunne DW. Helminth Allergens, Parasite-Specific IgE, and Its
639 Protective Role in Human Immunity. Frontiers in immunology 2014; 5:61.
640 61. Nakazawa T, Khan AF, Yasueda H, Saito A, Fukutomi Y, Takai T, Zaman K, Yunus M, Takeuchi
641 H, Iwata T, Akiyama K. Immunization of rabbits with nematode Ascaris lumbricoides antigens induces
642 antibodies cross-reactive to house dust mite Dermatophagoides farinae antigens. Bioscience,
643 biotechnology, and biochemistry 2013; 77:145-150.
644 62. Jin Y, Shen C, Huh S, Sohn WM, Choi MH, Hong ST. Serodiagnosis of toxocariasis by ELISA
645 using crude antigen of Toxocara canis larvae. Korean J Parasitol 2013; 51:433-439.
646 63. Nieuwenhuizen NE, Meter JM, Horsnell WG, Hoving JC, Fick L, Sharp MF, Darby MG, Parihar
647 SP, Brombacher F, Lopata AL. A cross-reactive monoclonal antibody to nematode haemoglobin
648 enhances protective immune responses to Nippostrongylus brasiliensis. PLoS Negl Trop Dis 2013;
649 7:e2395.
650 64. Noordin R, Smith HV, Mohamad S, Maizels RM, Fong MY. Comparison of IgG-ELISA and IgG4-
651 ELISA for Toxocara serodiagnosis. Acta tropica 2005; 93:57-62.
652 65. Levin ME, Le Souef PN, Motala C. Total IgE in urban Black South African teenagers: the
653 influence of atopy and helminth infection. Pediatric allergy and immunology : official publication of
654 the European Society of Pediatric Allergy and Immunology 2008; 19:449-454.

655

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	0.7
Age in years (median (range))	35 (10-63)	53 (39-63)	26 (10-45)	
BMI (kg/m2) 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	0.003 [a]
Cat ownership				
Early childhood	22.8	30.6	17.7	0.003
Late childhood	43.0	46.8	40.6	0.2
Current	21.6	21.3	21.7	0.9
Dog ownership				
Early childhood	11.8	8.9	13.7	0.2
Late childhood	28.6	27.6	29.2	0.7
Current	18.2	18.3	18.0	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	<0.001
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	<0.001*
Parental asthma	14.7	7.7	19.8	
Parental asthma not known	8.2	15.4	3.0	0.004
Wheeze, ever	18.3	24.7	13.9	0.005
Hay fever, ever	24.2	25.4	23.4	0.6
Rhinitis, ever	49.8	51.2	48.9	0.6
Asthma, ever	14.2	14.1	14.2	0.97
Eczema, ever	43.7	49.4	40.0	0.06
Any positive allergy test (specific IgE or SPT) [b]	39.3	31.1	44.5	0.006
IgE positivity[c]	32.7	19.5	41.2	<0.001
Total IgE in kU/L (mean (SD))	71.6 (193.9)	69.9 (240.9)	72.9 (156.7)	0.6
Total IgG4 (% positive) [d]			11.4	
Ascaris IgG4 (% positive)	17.9	29.2	10.3	<0.001
Toxocara IgG4 (% positive)	11.7	17.5	8.0	0.002

[a]Does not include students. [b] Positive IgE ≥0.35 kU/L and/or skin prick test positivity towards at least on of fur allergens (cat, timothy grass, birch and house dust mite). [c]Positive IgE ≥0.35 kU/L to at least one of four 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), hay fever, 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

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

	Parents (n=171)		**Offspring** (n=264)	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Gender (ref men)	0.69 (0.31, 1.55)	0.4	0.63 (0.26, 1.51)	0.3
Age	1.06 (0.99, 1.14)	0.7	**1.10 (1.03, 1.17)**	**0.009**
BMI (per kg/m2)	0.98 (0.88, 1.09)	0.7	**1.16 (1.06, 1.25)**	**<0.001**
Cat keeping				
Early childhood	1.42 (0.60, 3.39)	0.3	1.94 (0.61, 6.19)	0.2
Late childhood	1.53 (0.66, 3.52)	0.3	**6.25 (1.94, 20.12)**	**0.002**
Current	0.51 (0.17, 1.58)	0.3	0.70 (0.22, 2.22)	0.5
Dog ownership				
Early childhood	1.35 (0.35, 5.22)	0.9	1.50 (0.39, 5.76)	0.5
Late childhood	0.90 (0.35, 2.32)	0.8	1.63 (0.57, 4.68)	0.4
Current	0.44 (0.13, 1.56)	0.2	0.86 (0.29, 2.62)	0.8
Any pets from birth to age 15	1.79 (0.70, 4.53)	0.2	**6.15 (1.37, 27.54)**	**0.02**
Place of upbringing				
City or suburb (ref)		0.06		0.6
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)		-	

Parents=ECRHS; Offspring=RHINESSA

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

	Parents (n=171)		**Offspring** (n=264)	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Gender (ref men)	0.83 (0.43, 1.60)	0.6	0.55 (0.25, 1.21)	0.2
Age	1.07 (1.01, 1.13)	**0.5**	1.06(1.00, 1.12)	0.08
BMI (per kg/m2)	0.99 (0.91, 1.09)	**0.9**	**1.17 (1.08, 1.26)**	**<0.001**
Cat keeping				
Early childhood	**2.32 (1.11, 4.84)**	0.04	1.21 (0.40, 3.65)	0.6
Late childhood	**2.02 (0.99, 4.15)**	0.06	1.76 (0.69, 4.44)	0.2
Current	0.52 (0.21, 1.29)	0.2	0.72 (0.25, 2.07)	0.5
Dog ownership				
Early childhood	1.54 (0.48, 4.89)	0.8	0.79 (0.16, 3.76)	0.8
Late childhood	0.77 (0.34, 1.74)	0.5	1.62 (0.63, 4.15)	0.3
Current	0.41 (0.15, 1.14)	0.09	1.91 (0.80, 4.58)	0.1
Any pets from birth to age 15	**2.75 (1.21, 6.25)**	**0.02**	1.94 (0.70, 5.35)	0.2
Place of upbringing				
City or suburb (ref)		0.04		0.4
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)**		-	

Parents=ECRHS; Offspring=RHINESSA

TABLE 3A: Adjusted odds ratio (aOR) and 95% CI for anti-*Toxocara* 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
Wheeze, ever	0.77 (0.28, 2.08)	**0.6**	**2.97 (1.45, 7.76)**	**0.03**
Asthma, ever	0.86 (0.27, 2.77)	0.8	1.24 (0.29, 5.19)	0.7
Hay fever, ever	0.97 (0.38, 2.49)	**1.0**	**4.03 (1.63, 9.95)**	**0.003**
Rhinitis, ever	0.61 (0.27, 1.37)	**0.2**	**3.06 (0.97, 9.72)**	**0.06**
Eczema, ever	1.02 (0.45, 2.32)	**1.0**	**2.89 (1.08, 7.76)**	**0.04**
Any positive allergy test[b]	0.56 (0.21, 1.48)	0.2	1.22 (0.52, 2.88)	0.6
Any IgE positive[c]	0.76 (0.26, 2.19)	0.6	1.84 (0.78, 4.38)	0.2
Cat SPT/IgE positive[d]	0.72 (0.15, 3.46)	**0.7**	**5.65 (1.92, 16.6)**	**0.002**
HDM SPT/IgE positive[e]	0.46 (0.10, 2.13)	0.3	1.41 (0.52, 3.81)	0.5
Timothy grass SPT/IgE positive[f]	1.00 (0.31, 3.24)	1.0	2.12 (0.85, 5.33)	0.1

[a]Adjusted for: gender, age, bmi. [b] Positive IgE ≥0.35 kU/L and/or skin prick test positivity towards at least on of four allergens (cat, timothy grass, birch and house dust mite). [c]Positive IgE ≥0.35 kU/L to at least one of four 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. [e] Positive IgE ≥0.35 kU/L and/or skin prick test positivity towards house dust mite (*Dermatophagoides pteronyssinus* or *D. farinae*). [f]Positive IgE ≥0.35 kU/L and/or skin prick test positivity towards timothy grass. Parents=ECRHS. Offspring=RHINESSA

TABLE 3B: Adjusted odds ratio (aOR) and 95% CI for 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
Wheeze, ever	0.72 (0.31, 1.64)	0.4	1.81 (0.70, 4.68)	0.2
Asthma, ever	0.94 (0.36, 2.46)	0.9	0.47 (0.10, 2.25)	0.3
Hay fever, ever	1.60 (0.75, 3.41)	0.2	**3.50 (1.42, 8.63)**	**0.007**
Rhinitis, ever	0.87 (0.45, 1.71)	0.7	2.11 (0.82, 5.40)	0.1
Eczema, ever	0.83 (0.42, 1.65)	0.6	2.06 (0.85, 5.98)	0.1
Any positive allergy test[b]	0.80 (0.38, 1.70)	0.6	1.16 (0.50, 2.69)	0.7
Any IgE positive[c]	0.45 (0.17, 1.18)	0.1	1.66 (0.71, 3.86)	0.2
Cat SPT/IgE positive[d]	0.62 (0.16, 2.33)	0.5	**3.31 (1.19, 9.17)**	**0.02**
HDM SPT/IgE positive[e]	0.75 (0.26, 2.19)	0.6	1.29 (0.48, 3.49)	0.6
Timothy grass SPT/IgE positive[f]	1.32 (0.52, 3.39)	0.6	**2.66 (1.10, 6.47)**	**0.03**

[a]Adjusted for: gender, age, BMI. [b] Positive IgE ≥0.35 kU/L and/or skin prick test positivity towards at least on of fur allergens (cat, timothy grass, birch and house dust mite). [c]Positive IgE ≥0.35 kU/L to at least one of four 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 (*Dermatophagoides pteronyssinus* et *farinae*). [f] Positive IgE ≥0.35 kU/L and/or skin prick test positivity towards timothy grass. Parents=ECRHS. Offspring=RHINESSA

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	Paternal line		Maternal line	
	(n=264)	Sons (n=71)	Daughters (n=69)	Sons (n=69)	Daughters (n=48)
	aOR[a*] (95% CI)	aOR[a] (95% CI)	aOR[a] (95% CI)	aOR[a] (95% CI)	aOR[a] (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 [b]	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[c]	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 [d]	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 [e]	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 [f]	**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. [a]Adjusted for: offspring age, parent age. [b] Positive IgE ≥0.35 kU/L and/or skin prick test positivity towards at least on of fur allergens (cat, timothy grass, birch and house dust mite), also associated with parental allergy. [c]Positive IgE ≥0.35 kU/L to at least one of four 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 (*Dermatophagoides pteronyssinus* et *farinae*). [f] Positive IgE ≥0.35 kU/L and/or skin prick test positivity towards timothy grass. Parents=ECRHS. Offspring=RHINESSA. Gender missing for 3 offspring

FIGURE LEGEND:

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

Figure 1

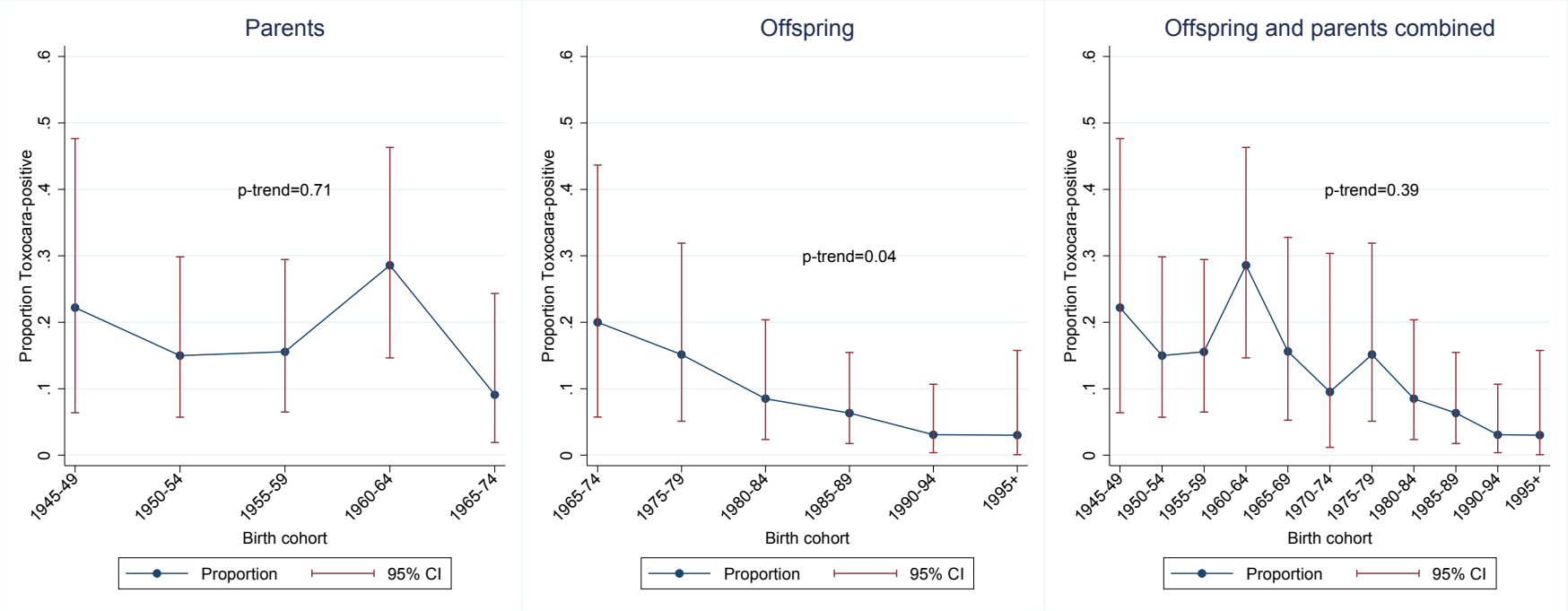
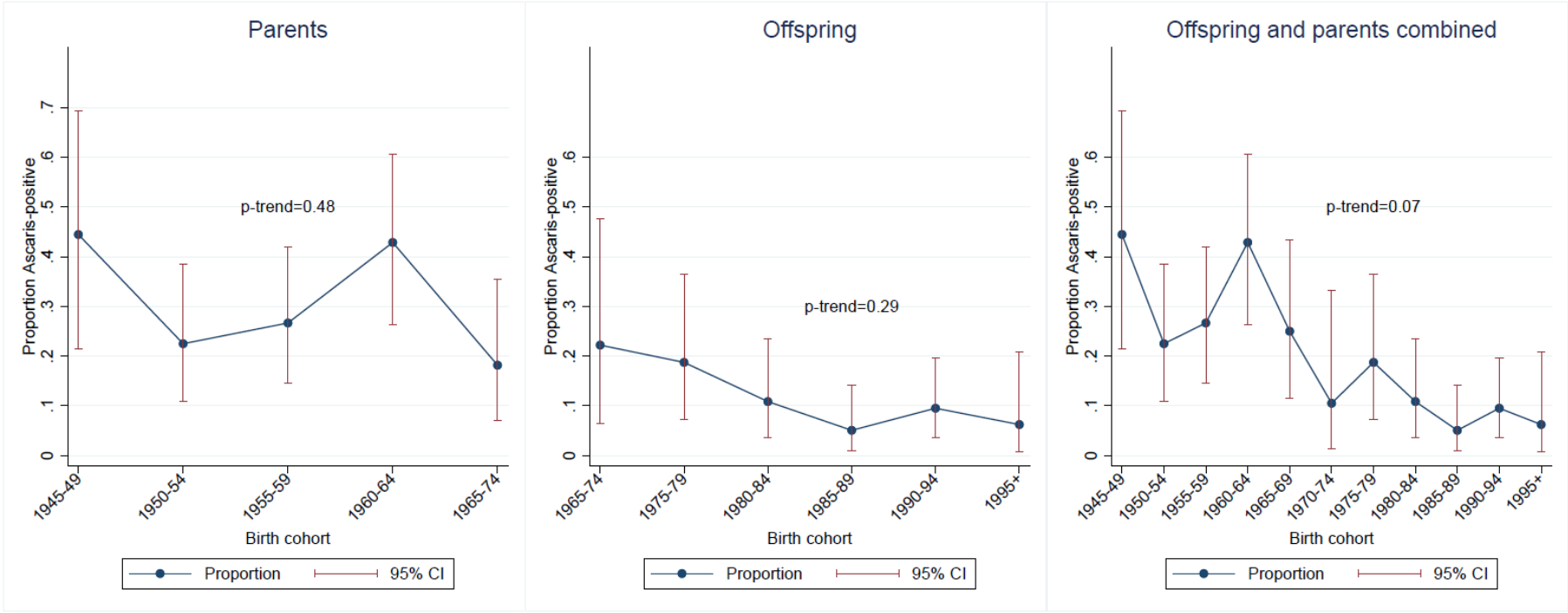

Parents
Proportion Toxocara-positive
0
.1
.2
.3
.4
.5
.6
p-trend=0.71
1945-49
1950-54
1955-59
1960-64
1965-74
Birth cohort
Proportion
95% CI
Offspring
Proportion Toxocara-positive
0
.1
.2
.3
.4
.5
.6
p-trend=0.04
1965-74
1975-79
1980-84
1985-89
1990-94
1995+
Birth cohort
Proportion
95% CI
Offspring and parents combined
Proportion Toxocara-positive
0
.1
.2
.3
.4
.5
.6
p-trend=0.39
1945-49
1950-54
1955-59
1960-64
1965-69
1970-74
1975-79
1980-84
1985-89
1990-94
1995+
Birth cohort
Proportion
95% CI

Figure 2


Parents
Proportion Ascaris-positive
0
.1
.2
.3
.4
.5
.6
.7
p-trend=0.48
1945-49
1950-54
1955-59
1960-64
1965-74
Birth cohort
Proportion
95% CI
Offspring
Proportion Ascaris-positive
0
.1
.2
.3
.4
.5
.6
p-trend=0.29
1965-74
1975-79
1980-84
1985-89
1990-94
1995+
Birth cohort
Proportion
95% CI
Offspring and parents combined
Proportion Ascaris-positive
0
.1
.2
.3
.4
.5
.6
p-trend=0.07
1945-49
1950-54
1955-59
1960-64
1965-69
1970-74
1975-79
1980-84
1985-89
1990-94
1995+
Birth cohort
Proportion
95% CI