

MS. JACQUELINE JANSE (Orcid ID : 0000-0003-4884-5881)

Received Date : 31-Mar-2016

Revised Date : 23-Dec-2016

Accepted Date : 24-Dec-2016

Article type : Original Article: Epidemiology and Genetics

Editor : Hans-Uwe Simon

***Opisthorchis felineus* negatively associates with skin test reactivity in Russia – EuroPrevall-
INCO study**

Olga S. Fedorova, PhD^{a*}, Jacqueline J. Janse, MSc,^{b*} Ludmila M. Ogorodova, PhD^a, Marina M. Fedotova, PhD^a, Rogier A. Achterberg, MSc^b, Jaco J. Verweij, PhD^{b#}, Montserrat Fernández-Rivas, MD, PhD^c, Serge A. Versteeg, BSc^d, James Potts, BSc^e, Cosetta Minelli, PhD^e, Ronald van Ree, PhD^{d,f}, Peter Burney, PhD^e, Maria Yazdanbakhsh, PhD^b

^aDepartment of Faculty Pediatrics, Siberian State Medical University, Tomsk, Russian Federation;

^bDepartment of Parasitology, Leiden University Medical Center, Leiden, the Netherlands; [#]Present address: Laboratory for Medical Microbiology and Immunology, St Elisabeth Hospital, Tilburg, the

Netherlands; ^cServicio de Alergia, Hospital Clínico San Carlos, IdISSC, Madrid, Spain; ^dDepartment of

Experimental Immunology, Academic Medical Center, Amsterdam, the Netherlands; ^eDepartment of Respiratory Epidemiology and Public Health, National Heart and Lung Institute, Imperial College

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13120

This article is protected by copyright. All rights reserved.

London, London, UK; ^fDepartment of Otorhinolaryngology, Academic Medical Center, Amsterdam, the Netherlands.

*These authors contributed equally

Short title

Opisthorchiasis and allergy in Russia

Corresponding author

Prof. Dr. M. Yazdanbakhsh

Department of Parasitology

Leiden University Medical Center

Albinusdreef 2

2333 ZA Leiden

The Netherlands

Tel.: +31 71 5265067

Fax: +31 71 5266907

E-mail: m.yazdanbakhsh@lumc.nl

Declaration of all sources of funding

Supported by EuroPrevall (FP6-FOOD-CT-2005-514000)

Abstract

Background: Most studies on the relationship between helminth infections and atopic disorders have been conducted in (sub)tropical developing countries where exposure to multiple parasites and lifestyle can confound the relationship. We aimed to study the relationship between infection with the fish-borne helminth *Opisthorchis felineus* and specific IgE, skin prick testing and atopic symptoms in Western-Siberia, with lifestyle and hygiene standards of a developed country.

Methods: Schoolchildren aged 7 to 11 years were sampled from one urban and two rural regions. Skin prick tests (SPT) and specific IgE (sIgE) against food and aeroallergens were measured and data on allergic symptoms and on demographic and socio-economic factors were collected by questionnaire. Diagnosis of opisthorchiasis was based on PCR performed on stool samples.

Results: Of the 732 children included, 34.9% had opisthorchiasis. The sensitisation to any allergen when estimated by positive SPT was 12.8%, while much higher, 24.0%, when measured by sIgE. Atopic symptoms in the past year (flexural eczema and/or rhinoconjunctivitis) were reported in 12.4% of the children. SPT was positively related to flexural eczema and rhinoconjunctivitis, but not to wheezing. Opisthorchiasis showed association with lower SPT response, as well as borderline association with low IgE reactivity to any allergen. However, the effect of opisthorchiasis on SPT response was not mediated by IgE, suggesting that opisthorchiasis influences SPT response through another mechanism. Opisthorchiasis also showed borderline association with lower atopic symptoms.

Conclusions: There is a negative association between a chronic helminth infection and skin prick test reactivity even in a developed country.

Key words

atopic symptoms, Europrevall, *Opisthorchis felineus*, Russia, skin prick testing

Abbreviations used

TC: Tomsk city

RL: Rural regions with low prevalence of reported clinical disease of opisthorchiasis

RH: Rural regions with high prevalence of reported clinical disease of opisthorchiasis

OR: Odds ratio

SPT: Skin prick testing

slgE: Specific IgE

INTRODUCTION

The prevalence of skin test reactivity and allergic diseases is higher in developed than in developing countries (1, 2) and within the same country, higher in urban than in rural area (3-6). Some of these observations might be explained by differences in exposure to environmental factors, like parasites. Helminth infections are associated with induction of Th2 responses and high levels of IgE. However

high levels of allergen specific IgE do not trigger SPT, as the IgE seems to be directed at carbohydrate moieties and of poor biological activity (7). Therefore the epidemiological studies of the association between helminths and allergies focus on SPT and reported symptoms as allergic outcomes. The association between helminths and SPT and allergic diseases has been studied with majority showing negative association for SPT and inconsistent results for symptoms (8). However, most studies were conducted in (sub)tropical regions where infection with multiple worms is common, and where lifestyle can be a confounding factor (9, 10). Therefore it is often difficult to dissect the association between allergies and parasites from the complex interplay of other environmental, socio-economic, and demographic influences.

Infection with the liver flukes of the family Opisthorchiidae has not been investigated in detail in the context of allergies. It is estimated that in European and Asian countries 17 million people are infected, of which at least 1.6 million with *Opisthorchis felineus*, the most common cause of opisthorchiasis in Russia and Europe (11). The life cycle of *O. felineus* includes freshwater snails as intermediate hosts, and freshwater fish as second intermediate host. People acquire the infection through eating raw or undercooked fish. Other final definitive hosts of this helminth can be carnivorous animals, like cats and dogs, that serve as reservoirs of infection (12, 13).

One of the areas in Russia with a high prevalence of *O. felineus* is Western Siberia. This region has the lifestyle and climate of a developed country, compared to (sub)tropical areas in developing countries, where multitude of helminth infections are usually highly prevalent. Therefore, this area provides a unique opportunity to address the question of whether helminth infections, within a more western lifestyle and therefore not confounded by factors present in endemic developing countries, are associated with atopic disorders.

Here, we examined the association of chronic *O. felineus* infection with skin prick tests (SPT) and IgE reactivity against food and aeroallergens and self-reported atopic symptoms in schoolchildren from three areas of Western Siberia.

METHODS

Study population and design

The study was part of the EuroPrevall project, a large EU-funded project on food allergy across Europe (14), and across Ghana (7) and China, India and Russia (EuroPrevall-International Cooperation (INCO)) as described before (15).

Participants were sampled from three areas of the Tomsk Oblast (Western Siberia, Russia, Figure 1): Tomsk city as urban area, and two rural regions with low or high reported prevalence of clinical disease of opisthorchiasis. Tomsk city (TC) is the capital of the Tomsk Oblast and it has a typical western lifestyle, with high degree of scientific and technical development. The rural region with low prevalence of opisthorchiasis (RL) is located around Tomsk city. Agriculture and forestry are well-developed industries in this region. The rural region with high prevalence of opisthorchiasis (RH) is located on the River Ob where fishing is the major occupation and river fish is a traditional food. Official medical statistics (2009) show that the incidence of clinical disease of opisthorchiasis is about 40, 44 and 109 new cases per 1,000 children per year, in respectively TC, RL and RH. These statistics are based on a unified system of official reports about treatment and diagnosis of patients for all clinics and hospitals of the Russian Federation. Diagnosis was based on clinical symptoms and the presence of eggs in stool samples (by microscopy) and/or presence of eggs or adult parasites in bile after duodenal intubation.

Our study design has multiple stages and starts with a cross-sectional survey of the general population (Figure 2), where children aged 7 to 11 years old were screened for self-reported adverse reactions to foods. Short standardized screening questionnaires (SQ, in Supplement) were distributed through schools and were completed by a parent or guardian. An extended case-control questionnaire (CCQ) included questions on allergic symptoms, and potential risks and exposures. A

trained interviewer administered the CCQ to a parent or guardian in a subsample of the children.

Skin-prick testing (SPT) and blood collection for serum specific IgE measurements was performed in all children with CCQ data (15). Of these 1,288 children, 732 gave a stool sample to determine the presence of *Opisthorchis felineus*.

The study was approved by the Siberian State Medical University Local Ethics Committee, Health Care Department, and Educational Department of the Tomsk Oblast Government. All parents or guardians of the children gave informed consent.

Skin prick testing

Skin prick tests (SPT) were done with reagents kindly provided by ALK-Abelló (Madrid, Spain), comprising 13 foods and 9 inhalants (Supplementary Table S1) (15). Tests were performed according to EAACI guidelines (16). Histamine (10 mg/ml) and saline were used as positive and negative controls, respectively. Valid and comparable results were obtained by carefully training three allergists to perform tests in the same way. Skin prick tests were considered positive if the mean wheal diameter was equal to or greater than 3 mm. We used SPT sensitisation to any allergen as composite outcome, which was defined as having at least one positive SPT to one of the foods or inhalant allergen sources.

Specific IgE

Specific IgE (sIgE) in serum was determined on an ImmunoCAP 250 System (Thermo Fisher Scientific, Uppsala, Sweden) to 24 food allergens and to 6 inhalants (Supplementary Table S1). Sera were first tested on 5 food mixtures (15) and positive samples were then tested against the individual foods of that mix. Specific IgE sensitisation to any allergen was defined by positive serology to at least one of the foods or inhalant allergen sources. sIgE results were considered positive when we detected levels of ≥ 0.35 kU_A/L.

Atopic symptoms

We used data from the case-control questionnaire to define self-reported symptoms in the past 12 months: wheezing (wheezing or whistling in the chest), rhinoconjunctivitis (problem with sneezing, or a runny, or a blocked nose without a cold or the flu, accompanied by itchy-watery eyes), and flexural eczema (itchy rash which was coming and going for at least 6 months, affecting any of the following places: the folds of the elbows, behind the knees, in front of the ankles, or around the neck, ears, or eyes). Symptoms associated to any SPT were combined in the composite outcome for atopic symptoms, which were rhinoconjunctivitis and flexural eczema and not wheezing.

Opisthorchis felineus – DNA isolation and real-time PCR

Diagnosis of opisthorchiasis was determined with real-time PCR on DNA of stool samples, performed at the Leiden University Medical Centre (the Netherlands).

Details are described in the additional Methods information in the Supplement. Ct values were used to determine the intensity of infection with arbitrary cut-offs: low/moderate infection if $Ct \geq 30$; and high infection if $Ct < 30$ (17). For analysis with the binary variable for opisthorchiasis, children with low/moderate and high DNA load are grouped together indicating infection with *O. felineus*.

Statistical analyses

Sampling weights were used to correct the analyses for the oversampling of children with adverse reactions to foods (Figure 2) (18, 19). The chance of participating in the study was predicted for each stage of the study design, and inverse probabilities were used to calculate non-response weights for each child. Final weighting factors incorporated design weights (inverse of the sampling fraction) and

two non-response weights (Supplementary Figure S1) corresponding to non-response to stage 2 - case control questionnaire and stage 3 - stool sample collection (Figure 2).

χ^2 -tests and logistic regression were used to analyse the data, guided by a conceptual framework (Figure 3). This framework shows the relations between *Opisthorchis felineus*, SPT, sIgE, and atopic symptoms. The association of *O. felineus* infection with sIgE and SPT to food and aeroallergens and atopic symptoms was investigated. To increase statistical efficiency (20), three composite outcomes were used in the main analyses: SPT and sIgE to any allergen (food and/or aero), and any atopic symptom in the past 12 months, which included flexural eczema and/or rhinoconjunctivitis. Main multivariate analyses of these associations used *a priori* adjustment for area, age and gender. As sensitivity analyses, we added to the models additional variables associated with sIgE, SPT and atopic symptoms in univariate analyses ($P < 0.05$). We tested for heterogeneity of odds ratios across study areas by adding interaction terms into the models. A P-value below 0.05 was considered statistically significant. Analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp) and STATA 11 software (StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP).

RESULTS

Study characteristics

A total of 732 children from the 3 areas participated in this study: 270 from Tomsk city (TC), 202 from the rural region with low prevalence of opisthorchiasis (RL) and 260 from the rural region with high prevalence of opisthorchiasis (RH) (Figure 2). Opisthorchiasis was found in 34.9% of the children and differed significantly among the three areas. The prevalence and intensity of opisthorchiasis infection were highest in RH and lowest in TC (Table 1).

All children with a negative SPT reaction (< 3 mm) to histamine ($n = 137$) were excluded from the SPT analysis. Histamine negativity occurred more frequently in the group of SPT-negative children compared to the SPT-positives to any allergen (20.3% vs. 10.3%, $P = 0.012$), in sIgE-negative children (to aero allergens) (20.7% vs. 11.9, $P = 0.015$) and in children from RL (25.7% vs. 14.1% in TC and 18.1% in RH, $P = 0.042$) (Supplementary Table S2). Histamine negatives were less likely to have mothers with university level (14.6%), compared to secondary (20.0%) or less than secondary education (25.5%), although statistical significance was borderline ($P = 0.052$). Important to note that three people performed all SPTs, but the percentage of children with a negative histamine reaction did not differ among them (19.5%, 16.8% and 18.2%, $P = 0.702$).

Of the remaining 595 children, 12.8% had a positive SPT response to at least one of the food (3.3%) or aero allergens tested (11.7%) (Table 1 and Supplementary Table S1). The prevalence of allergic symptoms in the past 12 months was 12.4% (7.4% for rhinoconjunctivitis and 5.9% for flexural eczema).

For 29 of the 732 children, sIgE could not be measured due to either refusal to donate blood or insufficient volume of serum. Of them, 24 children had reliable SPT data, so 571 children had data on both SPT and sIgE. All 29 children came from the rural regions (17 from RL, 12 from RH), but differences in opisthorchiasis and other variables were not observed between children with and without data on sIgE (data not shown). Of the 703 children with sIgE data, 24.0% had a positive sIgE to either one of the food (13.0%) or aero allergens (19.6%) (Table 1 and Supplementary Table S1).

Association between sIgE, SPT and symptoms

Specific IgE and SPT to food, aero and any allergen were associated strongly and significantly (Supplementary Table S3). Of the three self-reported symptoms in the past 12 months, rhinoconjunctivitis and flexural eczema, but not wheezing, were significantly associated to SPT to

any allergen. SPT reactivity among the symptomatic children was 43.5% for rhinoconjunctivitis, 33.8% for flexural eczema and 13.9% for wheezing. Therefore, the composite outcome for atopic symptoms in the past 12 months combined only rhinoconjunctivitis and flexural eczema.

Factors associated with opisthorchiasis and outcomes

Univariate associations of each demographic and socio-economic factor with opisthorchiasis and outcomes are presented in Table 2 (composite outcomes: SPT and sIgE to any allergen, atopic symptoms), Supplementary Table S4 (SPT and sIgE to food and aero allergens) and Table S5 (wheezing, rhinoconjunctivitis, flexural eczema). The rural areas, (less than) secondary education, overcrowding, past cat/dog ownership and current cat/dog ownership were positively associated with *O. felineus* infection.

Negative associations were found between female gender, low maternal education and sIgE to any allergen, between RL area, overcrowding, past and current cat/dog ownership and SPT to any allergen, and between RH area, (less than) secondary maternal education and atopic symptoms ($P < 0.05$, Table 2).

Opisthorchiasis and IgE

Total IgE levels were not significantly different between children with or without opisthorchiasis (Geometric Mean (GM) = 41.9 IU/mL, CI = 10.0–166.5 and GM = 49.3, CI = 11.4–203.2, $P = 0.326$, with *a priori* adjustment for area, age and gender). Opisthorchiasis was not significantly associated with sIgE to allergens, although there was a negative trend (any allergen: OR = 0.62, CI = 0.37–1.05, $P = 0.078$, with *a priori* adjustment, Table 3).

Opisthorchiasis and SPT

The presence of *O. felinus* was significantly associated with less SPT reactivity (OR = 0.47, CI = 0.23–0.94, $P = 0.034$, with *a priori* adjustment, Table 3 and in Supplementary Table S6 the results are shown when including all individuals i.e. including histamine negatives). sIgE to any allergen was then added to the model to investigate whether sIgE is involved in the causal pathway (Figure 3). This did not seem to be the case, given that the odds ratio was very similar though it lost statistical significance (OR = 0.51, CI = 0.20–1.32, $P = 0.165$). As sensitivity analysis, we also looked at the impact of further adjustment with variables associated with SPT in univariate analyses. This analysis resulted in very similar odds ratios although the P-values tended to be slightly higher (Table 3).

Opisthorchiasis and reported symptoms

There was a trend of less atopic symptoms in the presence of *O. felinus* (OR = 0.54, CI = 0.27–1.05, $P = 0.068$, with *a priori* adjustment). SPT was involved in the causal pathway between opisthorchiasis and atopic symptoms (Figure 3). When adding SPT to any allergen to the *a priori* model, the odds ratio changed to 1.02 (CI = 0.49–2.11, $P = 0.960$), while SPT was significantly associated to atopic symptoms (OR = 5.8, CI = 2.6–13.1, $P < 0.001$). As sensitivity analysis, we adjusted the *a priori* model further with maternal education. The P-value increased but the OR estimate changed only slightly (OR = 0.62, CI = 0.31–1.22, $P = 0.166$, Table 3).

DISCUSSION

Numerous studies have shown a negative association between helminth infections and SPT, but most, if not all, were conducted in developing countries (21, 22). In such countries it is often difficult to disentangle the presence of helminths from poor sanitation (frequent exchange of microorganisms) and non-western lifestyle that can also protect against atopy. The presence of *O.*

felineus infection in the Western-Siberian Tomsk Oblast provides an opportunity to study a helminth infection in an area with a more western lifestyle. This study showed a negative association between opisthorchiasis and SPT to any allergen allowing us to establish that a chronic helminth infection is associated with less skin prick test reactivity even in a developed country.

In affluent countries, increased exposure to microorganisms is associated with lower specific IgE and therefore with lower SPT (23). In the tropical regions where multiple helminth infections are highly prevalent, elevated total IgE levels are measured along with elevated specific IgE to allergens. For example a study in Indonesia showed total IgE levels to be around 1900-4100 IU/ml, and that 83.4% of the schoolchildren had sIgE to HDM or cockroach, whereas only 25.7% were positive in SPT to these allergens (9). The negative association found between helminth infections and SPT in tropical regions is not reflected in sIgE. In other words, despite high levels of IgE to allergens, the SPT is lower in helminth infected subjects (9, 24). Indeed, the odd ratios for being SPT positive, if IgE positive to the same allergen, is much lower in developing countries than in affluent countries (24). The situation appears to be different in Western-Siberia. First of all, whereas 24.1% of subjects had IgE to any allergens, this was 12.8% in SPT reactivity and although this is not showing a one to one translation of IgE to SPT, it is not as extreme as what is seen in tropical regions (7, 9). Moreover, in our study, *O. felineus* infection does not seem to lead to extremely high total IgE levels (49.3 IU/mL), and even was associated with reduced specific IgE to allergens, although this did not reach statistical significance. Another difference to note is that the association between sIgE and SPT is very strong (OR >25), which is much higher than what is seen in tropical areas (24). This could be due to the paucity of other helminth infections in Siberia or less cross reactivity between antigens of *O. felineus* and allergens studied; less than described for cross reactivity between schistosomes or filaria and peanut or tropomyosin allergens (7, 25). Together, these data indicate that although exposure to *O. felineus* is associated with less SPT, this is not via a reduction in specific IgE as confirmed also by the analysis which showed that sIgE did not affect the association between *O. felineus* and SPT. Therefore the situation in Western-Siberia is unlike what is seen in other developed countries (23).

The negative association could be explained by suppressory network induced by *O. felineus*, directly affecting SPT. This is one of the mechanisms that has been proposed to mediate the lower SPT in helminth infected subjects in the tropical countries.

A negative association was found between *O. felineus* infection and atopic symptoms, although this did not reach statistical significance. Previous studies on allergic symptoms and other helminth infections conducted in Ecuador, Cuba and Norway have shown inconsistent results (26-29). This indicates that the association between helminths and reported symptoms of atopy are less clear cut and could also be affected by the use of questionnaires, which are prone to bias in results.

The International Study of Asthma and Allergies in Childhood (ISAAC) has investigated the link between SPT sensitivity and allergic symptoms in affluent and non-affluent countries. Comparing the prevalences with those in ISAAC phase III similar percentages for rhinoconjunctivitis and flexural eczema were found, but prevalence for wheezing was higher (18.0% versus 11.4% in 6-7 year old or 11.2% in 13-14 year old children) (1). In affluent countries, both rhinoconjunctivitis (30) and flexural eczema were positively correlated to SPT (31), as found in our study population. Contrary to our results, a positive association was also found for wheezing, especially in affluent countries (2). It is not clear why in this region SPT is not associated with wheeze but it is possible that *Opisthorchis* infection interferes with the association, as described for hookworm infection (32).

An unexpected finding of this study was the high percentage of children who had a negative histamine reaction, almost 19% (n = 137), who were excluded from analyses of SPT. When we repeated the analyses including these children, the conclusion did not change. We used standardized extracts and protocols and assessed trained personnel competency based on inter assay reproducibility when measuring SPT reactivity. Moreover, the use of anti-histamines could not explain these results. In a study of 9-year-old children from Italy, Poland and Libya, mean wheal diameters elicited by 10 mg/ml histamine followed a gradient with lowest diameter in Poland (3.75 mm), then Italy (4.43 mm) and highest in Libya (5.06 mm) (33). The mean diameter of histamine SPT

in our study participants was very similar to that seen in Poland (3.68 mm). As the geographic coordinates of Poland and Russia are comparable (52°N for Poland and 56°N for Tomsk), our results indicate that further studies are needed to investigate what factors determine negative histamine reaction.

Interestingly, cat/dog ownership, currently as well as in the first two years of life, were positively associated with opisthorchiasis. Domestic cats and dogs are involved in the life cycle and transmission (11), and therefore could intensify infections in humans. The cross-sectional study design is thus a limitation, but our study also has a number of strengths. The same standardized methodology and laboratory measurements were used in all three areas, allowing valid comparisons to be made. This is the first study providing data on *O. felinus* infection intensity, as PCR on stool samples has been used to determine opisthorchiasis. Furthermore, this method of diagnosis is much more sensitive than detection of eggs in stool samples.

In conclusion, this first large scale study conducted in an area with a more western lifestyle and high sanitation standards but with prevalent chronic systemic helminth infection, has shown a negative association between *O. felinus* infection and SPT to any allergen, and – although statistical significance is not reached – a trend for a negative association with sIgE and atopic symptoms. The areas in Western Siberia where opisthorchiasis is endemic could provide unique opportunities to find the mechanisms underlying the suppressive effects of helminths on atopy.

Acknowledgements

The authors wish to thank all children and parents in taking part in the EuroPrevall-INCO surveys. We also thank all fieldworkers who gathered the data in the study areas. We thank Domingo Barber and Lucia Jimeno of ALK-Abelló for kindly providing the SPT reagents. The authors declare no conflict of interest and funding.

Authors' contributions

OSF conducted the study in Tomsk and reviewed the manuscript; JJJ carried out the statistical analysis and drafted the paper; LMO supervised the study in Tomsk; MMF collected stool samples; RAA isolated DNA from stool samples in Tomsk; JJV diagnosed opisthorchiasis with PCR and reviewed the manuscript; MFR supervised the clinical aspects of the study and reviewed the manuscript; SAV measured IgE antibodies; JP cleaned and prepared the database; CM advised on statistical analysis and reviewed the manuscript; RvR supervised the study, advised on allergic parameters, and reviewed the manuscript; PB supervised epidemiological design and training of the investigators, advised on statistical analysis, and reviewed the manuscript; MY supervised the study, the data analysis and reviewed the manuscript. All authors have read and approved the final manuscript.

Figure legends

Figure 1. Map showing Russia (upper) and districts and river Ob in Tomsk Oblast (lower).

Figure 2. Consort diagram. *TC*: Tomsk city; *RL*: Rural region with low reported prevalence of clinical disease of opisthorchiasis; *RH*: Rural region with high reported prevalence of clinical disease of opisthorchiasis; *RR*: Response rate; **24 priority foods*: apple, banana, buckwheat, carrot, celery, corn, cow's milk, fish, hazelnut, hen's egg, kiwi, lentils, melon, mustard, peach, peanut, poppy seeds, sesame, shrimp, soy, sunflower seeds, tomato, walnut and wheat.

Figure 3. Conceptual framework.

REFERENCES

1. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 2006;**368**(9537):733-743.
2. Weinmayr G, Weiland SK, Bjorksten B, Brunekreef B, Buchele G, Cookson WO, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children. *Am.J.Respir.Crit Care Med.* 2007;**176**(6):565-574.
3. Shpakou A, Brozek G, Stryzhak A, Neviartovich T, Zejda J. Allergic diseases and respiratory symptoms in urban and rural children in Grodno Region (Belarus). *Pediatr.Allergy Immunol.* 2012;**23**(4):339-346.
4. Schram ME, Tedja AM, Spijker R, Bos JD, Williams HC, Spuls PI. Is there a rural/urban gradient in the prevalence of eczema? A systematic review. *Br.J.Dermatol.* 2010;**162**(5):964-973.
5. Majkowska-Wojciechowska B, Pelka J, Korzon L, Kozłowska A, Kaczala M, Jarzebska M, et al. Prevalence of allergy, patterns of allergic sensitization and allergy risk factors in rural and urban children. *Allergy* 2007;**62**(9):1044-1050.
6. Addo-Yobo EO, Woodcock A, Allotey A, Baffoe-Bonnie B, Strachan D, Custovic A. Exercise-induced bronchospasm and atopy in Ghana: two surveys ten years apart. *PLoS.Med.* 2007;**4**(2):e70.
7. Amoah AS, Obeng BB, Larbi IA, Versteeg SA, Aryeetey Y, Akkerdaas JH, et al. Peanut-specific IgE antibodies in asymptomatic Ghanaian children possibly caused by carbohydrate determinant cross-reactivity. *J.Allergy Clin.Immunol.* 2013;**132**(3):639-647.
8. Wammes LJ, Mpairwe H, Elliott AM, Yazdanbakhsh M. Helminth therapy or elimination: epidemiological, immunological, and clinical considerations. *The Lancet Infectious Diseases* 2014;**14**(11):1150-1162.
9. Hamid F, Wiria AE, Wammes LJ, Kaisar MM, Djuardi Y, Versteeg SA, et al. Risk Factors Associated with the Development of Atopic Sensitization in Indonesia. *PLoS.One.* 2013;**8**(6):e67064.
10. Supali T, Djuardi Y, Wibowo H, van Ree R, Yazdanbakhsh M, Sartono E. Relationship between different species of helminths and atopy: a study in a population living in helminth-endemic area in Sulawesi, Indonesia. *Int.Arch.Allergy Immunol.* 2010;**153**(4):388-394.
11. Pakharukova MY, Mordvinov VA. The liver fluke *Opisthorchis felinus*: biology, epidemiology and carcinogenic potential. *Trans R Soc Trop Med Hyg* 2016;**110**(1):28-36.
12. Pozio E, Armignacco O, Ferri F, Gomez Morales MA. *Opisthorchis felinus*, an emerging infection in Italy and its implication for the European Union. *Acta Trop.* 2013;**126**(1):54-62.
13. Mordvinov VA, Yurlova NI, Ogorodova LM, Katokhin AV. *Opisthorchis felinus* and *Metorchis bilis* are the main agents of liver fluke infection of humans in Russia. *Parasitol.Int.* 2012;**61**(1):25-31.
14. Mills EN, Mackie AR, Burney P, Beyer K, Frewer L, Madsen C, et al. The prevalence, cost and basis of food allergy across Europe. *Allergy* 2007;**62**(7):717-722.
15. Wong GW, Mahesh PA, Ogorodova L, Leung TF, Fedorova O, Holla AD, et al. The EuroPrevall-INCO surveys on the prevalence of food allergies in children from China, India and Russia: the study methodology. *Allergy* 2010;**65**(3):385-390.
16. Position paper: Allergen standardization and skin tests. The European Academy of Allergology and Clinical Immunology. *Allergy* 1993;**48**(14 Suppl):48-82.

17. Pillay P, Taylor M, Zulu SG, Gundersen SG, Verweij JJ, Hoekstra P, et al. Real-time polymerase chain reaction for detection of *Schistosoma* DNA in small-volume urine samples reflects focal distribution of urogenital Schistosomiasis in primary school girls in KwaZulu Natal, South Africa. *Am.J.Trop.Med.Hyg.* 2014;**90**(3):546-552.
18. Reilly M, Torrang A, Klint A. Re-use of case-control data for analysis of new outcome variables. *Stat.Med.* 2005;**24**(24):4009-4019.
19. Korn EL, Graubard BI. Epidemiologic studies utilizing surveys: accounting for the sampling design. *Am.J.Public Health* 1991;**81**(9):1166-1173.
20. Cordoba G, Schwartz L, Woloshin S, Bae H, Gotzsche PC. Definition, reporting, and interpretation of composite outcomes in clinical trials: systematic review. *BMJ* 2010;**341**:c3920.
21. Feary J, Britton J, Leonardi-Bee J. Atopy and current intestinal parasite infection: a systematic review and meta-analysis. *Allergy* 2011;**66**(4):569-578.
22. Flohr C, Quinnell RJ, Britton J. Do helminth parasites protect against atopy and allergic disease? *Clin.Exp.Allergy* 2009;**39**(1):20-32.
23. de Meer G, Janssen NA, Brunekreef B. Early childhood environment related to microbial exposure and the occurrence of atopic disease at school age. *Allergy* 2005;**60**(5):619-625.
24. Obeng BB, Amoah AS, Larbi IA, de Souza DK, Uh HW, Fernandez-Rivas M, et al. Schistosome infection is negatively associated with mite atopy, but not wheeze and asthma in Ghanaian schoolchildren. *Clin Exp Allergy* 2014;**44**(7):965-975.
25. Santiago HC, Bennuru S, Boyd A, Eberhard M, Nutman TB. Structural and immunologic cross-reactivity among filarial and mite tropomyosin: implications for the hygiene hypothesis. *J.Allergy Clin.Immunol.* 2011;**127**(2):479-486.
26. Cooper PJ, Chico ME, Bland M, Griffin GE, Nutman TB. Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. *Am.J.Respir.Crit Care Med.* 2003;**168**(3):313-317.
27. Cooper PJ. Interactions between helminth parasites and allergy. *Curr.Opin.Allergy Clin.Immunol.* 2009;**9**(1):29-37.
28. Wordemann M, Diaz RJ, Heredia LM, Collado Madurga AM, Ruiz EA, Prado RC, et al. Association of atopy, asthma, allergic rhinoconjunctivitis, atopic dermatitis and intestinal helminth infections in Cuban children. *Trop.Med.Int.Health* 2008;**13**(2):180-186.
29. Boas H, Tapia G, Rasmussen T, Ronningen KS. *Enterobius vermicularis* and allergic conditions in Norwegian children. *Epidemiol.Infect.* 2014;**142**(10):2114-2120.
30. Weinmayr G, Forastiere F, Weiland SK, Rzehak P, Abramidze T, Annesi-Maesano I, et al. International variation in prevalence of rhinitis and its relationship with sensitisation to perennial and seasonal allergens. *Eur.Respir.J.* 2008;**32**(5):1250-1261.
31. Flohr C, Weiland SK, Weinmayr G, Bjorksten B, Braback L, Brunekreef B, et al. The role of atopic sensitization in flexural eczema: findings from the International Study of Asthma and Allergies in Childhood Phase Two. *J.Allergy Clin.Immunol.* 2008;**121**(1):141-147.
32. Mpairwe H, Ndibazza J, Webb EL, Nampijja M, Muhangi L, Apule B, et al. Maternal hookworm modifies risk factors for childhood eczema: results from a birth cohort in Uganda. *Pediatric Allergy and Immunology* 2014;**25**(5):481-488.
33. Ronchetti R, Villa MP, Rennerova Z, Haluszka J, Dawi EB, Di FG, et al. Allergen skin weal/radioallergosorbent test relationship in childhood populations that differ in histamine skin reactivity: a multi-national survey. *Clin.Exp.Allergy* 2005;**35**(1):70-74.

Table 1 Distribution of opisthorchiasis (prevalence and intensity), SPT, symptoms, specific IgE, and other variables in the whole study population (N = 732, unless otherwise stated).

	n	%*
Opisthorchiasis:		
Presence ¹		
All areas	237	34.9
TC (N = 270)	44	20.8
RL (N = 202)	68	39.2
RH (N = 260)	125	49.3
Intensity of infection ¹		
Low/moderate infection		
All areas	110	16.5
TC (N = 270)	34	16.4
RL (N = 202)	30	15.6
RH (N = 260)	46	17.7
High infection		
All areas	127	18.4
TC (N = 270)	10	4.4
RL (N = 202)	38	23.6
RH (N = 260)	79	31.6
slgE, SPT, symptoms:		
slgE to any allergen² (N = 703)	181	24.0
slgE to food allergen ² (N = 703)	110	13.0
slgE to aero allergen ² (N = 703)	151	19.5
SPT to any allergen² (N = 595)	104	12.8
SPT to food allergen ² (N = 595)	40	3.3

SPT to aero allergen ² (N = 595)	96	11.7
Wheezing ³ (N = 731)	157	18.0
Atopic symptoms³	123	12.4
Rhinoconjunctivitis ³	72	7.4
Flexural eczema ³	59	5.9
Other variables:		
Girl	367	47.2
Maternal education (N = 724)		
University	267	33.1
Secondary	356	49.3
Less than secondary	101	17.6
Older siblings (N = 727)	264	37.8
Maternal atopy ⁴ (N = 703)	287	32.4
Antibiotic use ⁵ (N = 723)	397	47.9
Day-care ⁶ (N = 724)	219	29.7
Overcrowding ⁷ (N = 727)	30	4.8
Breastfeeding ⁸ (N = 724)	583	82.7
Past cat/dog ownership ⁵ (N = 728)	478	71.0
Current cat/dog ownership (N = 731)	506	71.1

*Weighted percentages are shown

¹Presence of infection Ct < 50. Intensity of infection: low/moderate 30 ≤ Ct < 50; high Ct < 30

²≥ 3 mm (SPT) or ≥ 0.35 kUA/L (sIgE) to at least one of the measured food, aero or any allergen(s)

³In the past year

⁴Food allergy, asthma, eczema/skin allergy, nasal allergy/hay fever ever

⁵In first two years of child's life

⁶Attendance of playschool or nursery with older children before age of two years

⁷Two or more children per sleeping room before age of two years

⁸For at least one month

Table 2 Univariate weighted models for opisthorchiasis, IgE and SPT to any allergen, and atopic symptoms in the past 12 months (flexural eczema and/or rhinoconjunctivitis)

Variables	Opisthorchiasis			IgE any			SPT any			Atopic symptoms		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Opisthorchiasis ¹	-			0.60	0.36	0.050	0.44	0.21	0.028	0.47	0.24	0.028
				-				-			-	
				1.00				0.91			0.92	
Area												
TC	1.00			1.00			1.00			1.00		
RL	2.46	1.41	0.002	0.66	0.36	0.175	0.29	0.12	0.008	0.79	0.41	0.501
		-			-			-			-	
		4.29			1.20			0.73			1.55	
RH	3.70	2.21	<.001	0.81	0.48	0.434	0.82	0.42	0.571	0.38	0.18	0.012
		-			-			-			-	
		6.21			1.38			1.62			0.81	
Age	1.11	0.92	0.258	1.02	0.83	0.846	1.06	0.80	0.701	1.10	0.85	0.464
		-			-			-			-	
		1.34			1.26			1.40			1.41	
Girl	1.23	0.82	0.323	0.62	0.38	0.049	0.70	0.37	0.267	0.96	0.53	0.880
		-			-			-			-	
		1.86			0.99			1.32			1.71	
Maternal education												
University	1.00			1.00			1.00			1.00		
Secondary	2.24	1.37	0.001	0.85	0.51	0.516	0.82	0.42	0.573	0.53	0.28	0.045
		-			-			-			-	
		3.65			1.40			1.62			0.98	
Less than secondary	3.04	1.60	0.001	0.37	0.16	0.024	0.63	0.23	0.363	0.29	0.11	0.014
		-			-			-			-	
		5.77			0.88			1.71			0.77	
Older siblings	1.39	0.91	0.124	1.33	0.82	0.252	1.28	0.67	0.455	0.94	0.51	0.854
		-			-			-			-	
		2.13			2.15			2.43			1.74	
Maternal atopy ²	0.84	0.54	0.443	1.24	0.76	0.391	1.72	0.91	0.097	1.76	0.97	0.063
		-			-			-			-	

	1.31			2.03			3.25			3.21		
Antibiotic use ³	0.71	0.47	0.106	1.36	0.84	0.207	1.38	0.73	0.320	1.23	0.68	0.491
		-			-			-			-	
		1.08			2.18			2.62			2.22	
Day-care ⁴	0.81	0.51	0.363	1.43	0.86	0.166	0.85	0.43	0.638	1.53	0.83	0.174
		-			-			-			-	
		1.28			2.35			1.69			2.81	
Overcrowding ⁵	4.03	1.45	0.007	1.16	0.37	0.798	0.10	0.02	0.004	0.45	0.07	0.414
		-			-			-			-	
		11.2			3.60			0.49			3.06	
Breastfeeding (>1month)	0.83	0.48	0.489	1.41	0.73	0.306	1.77	0.72	0.210	1.87	0.78	0.159
		-			-			-			-	
		1.42			2.71			4.32			4.46	
Past cat/dog ownership ³	2.26	1.37	0.001	0.70	0.43	0.166	0.45	0.23	0.014	1.06	0.56	0.856
		-			-			-			-	
		3.73			1.16			0.85			2.01	
Current cat/dog ownership	2.24	1.36	0.002	0.64	0.39	0.081	0.43	0.23	0.010	0.66	0.36	0.184
		-			-			-			-	
		3.69			1.06			0.82			1.22	
Past parental smoking ³	-			-			-			0.88	0.47	0.686
											-	
											1.63	
Current parental smoking	-			-			-			0.65	0.36	0.141
											-	
											1.16	

Odds ratios (OR), 95% confidence intervals (95% CI), P-values. Statistically significant results are shown in bold.

¹Infection Ct < 50

²Food allergy, asthma, eczema/skin allergy, nasal allergy/hay fever ever

³In first two years of child's life

⁴Attendance of playschool or nursery with older children before age of two years

⁵Two or more children per sleeping room before age of two years

Table 3 Adjusted weighted models for the association between opisthorchiasis and outcomes IgE, SPT, and (atopic) symptoms in the past 12 months

Outcomes	Main analyses*				Sensitivity analyses [‡]				Additional variables
	N	OR	95% CI	P	N	OR	95% CI	P	
IgE any	703	0.62	0.37 - 1.05	0.078	695	0.62	0.36 - 1.07	0.087	Maternal education
IgE food	703	0.74	0.38 - 1.41	0.357	695	0.80	0.40 - 1.59	0.519	Maternal education
IgE aero	703	0.62	0.35 - 1.09	0.094	695	0.63	0.35 - 1.12	0.114	Maternal education
SPT any	595	0.47	0.23 - 0.94	0.034	591	0.57	0.28 - 1.16	0.122	Overcrowding, past and current cat/dog ownership
SPT food	595	0.32	0.057 - 1.74	0.186	588	0.37	0.068 - 1.98	0.243	Breastfeeding, past cat/dog ownership
SPT aero	595	0.53	0.26 - 1.08	0.081	591	0.64	0.31 - 1.35	0.243	Overcrowding, past and current cat/dog ownership
Wheezing	731	1.14	0.67 - 1.94	0.638					
Atopic symptoms	732	0.54	0.27 - 1.05	0.068	724	0.62	0.31 - 1.22	0.166	Maternal education
Rhinoconjunctivitis	732	0.63	0.27 - 1.44	0.272	727	0.71	0.31 - 1.64	0.419	Overcrowding, current cat/dog ownership
Flexural eczema	732	0.45	0.18 - 1.14	0.093	728	0.45	0.18 - 1.16	0.098	Daycare

Odds ratios (OR), 95% confidence intervals (95% CI), P-values. Statistically significant results are shown in bold.

*A *priori* adjusted for area, age and gender

‡ Additionally adjusted for variables associated with outcomes in univariate analysis (Table 2 and Supplementary Table S4 & S5)





