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ORIGINAL PAPER

Seroepidemiology of human *Toxocara* and *Ascaris* infections in the Netherlands

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Abstract *Toxocara canis*, *Toxocara cati* and *Ascaris suum* are worldwide-distributed zoonotic roundworms of dogs, cats and pigs, respectively. The epidemiology of these parasites in developed countries is largely unclear. Two countrywide cross-sectional serosurveys were therefore conducted in the Netherlands in 1995/1996 and 2006/2007 to investigate the prevalence, trends and risk factors for human *Toxocara* and *Ascaris* infections in the general population. The Netherlands is characterized by high pig production, freedom from stray dogs and virtual absence of autochthonous infections with the human-adapted roundworm *Ascaris lumbricoides*. Over the 10 years between the two serosurveys, *Toxocara* seroprevalence decreased significantly from 10.7 % ($n = 1159$) to 8.0 % ($n = 3683$), whereas *Ascaris* seroprevalence increased significantly from 30.4 % ($n = 1159$) to 41.6 % ($n = 3675$), possibly reflecting concomitant improvements in pet hygiene management and increased exposure to pig manure-contaminated soil. Increased anti-*Toxocara* IgGs were associated with increasing age, male gender, contact with soil, ownership of cats, cattle or pigs, hay fever, low education, high income and non-Western ethnic origin. Increased anti-*Ascaris* IgGs were associated with increasing age, owning pigs, low education, childhood geophagia and non-Dutch ethnic origin. Besides identifying specific groups at highest risk of *Toxocara* and *Ascaris* infections, our results suggest that these infections mainly occur through environmental, rather than foodborne, routes, with direct contact with soil or cat and pig ownership being potentially modifiable exposures.

Keywords *Toxocara canis* · *Toxocara cati* · *Ascaris suum* · Seroprevalence · Risk factors · Trends

Lapo Mughini-Gras and Margriet Harms contributed equally to this work.

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Introduction

Toxocara canis, *Toxocara cati* and *Ascaris suum* are worldwide-distributed helminths with zoonotic potential whose final hosts among domestic animals are dogs, cats and pigs, respectively. The adult worms live in the gut of their final hosts and lay eggs that are dispersed into the environment through the hosts' faeces. Transmission to humans occurs mainly by ingestion of larvated eggs from faecally contaminated soil, water and vegetables that are not properly washed, cooked or peeled. Besides individuals occupationally/recreationally exposed to soil, those exhibiting pica or suffering from mental retardation, as well as those coming into contact with contaminated environments (as it is often the case of children's playgrounds after indiscriminate defecation by dogs and cats) (Uga and Kataoka 1995) are at high risk of infection (Despommier 2003; Kaplan et al. 2004). People may also acquire *Toxocara* infection by consuming raw/undercooked meat of potential paratenic hosts like chicken and sheep (Nagakura et al. 1989; Salem and Schantz 1992), and a recent review pointed out that the significance of paratenic hosts as sources of infection for definitive hosts is one of the biggest gaps in our understanding of *Toxocara* epidemiology (Holland 2015). Although it is theoretically possible that *A. suum* could be transmitted through consumption of raw/undercooked pork, this has not been proved. Yet, *A. suum* infection by consumption of raw/undercooked offal from a paratenic host like chicken has been deemed possible based on experiments on pigs (Permin et al. 2000), and the habit of regularly consuming raw bovine or porcine liver was described in some severely ill people infected with *A. suum* (Izumikawa et al. 2011; Kim et al. 2002).

Since humans are accidental hosts for *Toxocara* spp. and *A. suum*, their larvae usually fail to reach the adult stage and undertake aberrant migrations throughout the body. However, it has been reported that *A. suum* can develop to adult worms in humans (Nejsum et al. 2005), further stimulating the debate of whether *A. suum* (collected mainly from pigs) and *Ascaris lumbricoides* (infecting mainly humans) are truly two distinct species (Leles et al. 2012). Although human infection with tissue-dwelling larvae is frequently asymptomatic, they may damage whatever tissue they enter, resulting in a syndrome known as visceral larva migrans (VLM), a serious complication of which is the migration of the larvae in the eye causing ocular larva migrans (OLM) (Smith et al. 2009). Moreover, a positive association between seropositivity to *A. suum* and wheeze, asthma and food- and aero-allergen sensitization has been found in 4-year-old children in the Netherlands (Pinelli et al. 2009), supporting the hypothesis that low-level or transient infections with such helminths may promote allergic reactions. Several epidemiological and experimental studies reviewed elsewhere (Pinelli and Aranzamendi 2012) suggest that also *Toxocara* infections contribute to the development of allergic manifestations and exacerbation of airway inflammations.

As detection of *Toxocara* or *Ascaris* larvae in biopsies is rare and eggs are hard to find in human faeces in countries like the Netherlands (de Wit et al. 2001a, b), diagnosis of human *Toxocara* and *Ascaris* infections relies mainly on serology. We have previously reported that sera of Dutch patients ($n = 2838$) suspected of VLM/OLM referring to the Netherlands' National Institute for Public Health and the Environment (RIVM) for routine serodiagnosis of *Toxocara* and *Ascaris* infections during 1998–2009 had a seroprevalence (IgG) of 8 % for *Toxocara* and 33 % for *Ascaris* (Pinelli et al. 2011). However, these estimates refer to people selected based on clinical grounds. To determine the true magnitude of (the exposure to) these zoonotic helminths and to identify targets for control measures, population-based surveys and risk factor analyses are needed. In the Netherlands, two nationwide cross-sectional serosurveys were conducted, the so-called PIENTER-1 (1995–1996) (De Melker and Conyn-van Spaendonck 1998) and PIENTER-2 (2006–2007) (van der Klis et al. 2009) to establish a large serum bank with accompanying epidemiological information representative of the Dutch population. These serosurveys were primarily aimed not only to perform immunosurveillance to evaluate the Dutch national immunization programme but also to address additional research questions. This provides the unique opportunity to obtain insights into a multitude of infections occurring in the population, including those caused by *Toxocara* and *Ascaris*, whose epidemiology in developed countries is still largely unclear. Therefore, the aim of this study was to determine the seroprevalence and factors associated with increased or decreased IgG antibody levels for *Toxocara* and *Ascaris* in the general population of a high-income country like the Netherlands, which is characterized by high swine production, freedom from stray dogs and virtual absence of autochthonous *A. lumbricoides* infections.

Methods

Data collection

Data were collected from October 1995 to December 1996 (PIENTER-1) and from February 2006 to June 2007 (PIENTER-2). The design and rationale of both serosurveys are described in detail elsewhere (De Melker and Conyn-van Spaendonck 1998; van der Klis et al. 2009). In brief, a two-stage cluster sampling design, with municipalities nested in regions, was applied: Age-stratified random sampling (<1, 1–4, 5–9, …, 75–79 years) was performed in 48 municipalities within five study-defined geographical regions of approximately equal population size in the Netherlands. In total, 18,217 (PIENTER-1) and 24,147 (PIENTER-2) individuals were invited to participate. In 12 municipalities, an oversampling of the largest non-Western migrant groups in the Netherlands in those years (i.e. from Morocco, Turkey, Suriname and Netherlands Antilles) was performed in PIENTER-2; 2558 people were invited in this extra sample. Each invited individual received an invitation letter, a brochure introducing the study, a questionnaire, an informed consent form and a prescheduled appointment for serum sample donation. The questionnaire contained questions regarding demographic characteristics, medical history, activities and behaviours putatively related to infectious disease transmission (e.g. foreign travel, occupation, eating habits, etc.). Informed consent was obtained for all participants. Information on socio-economic status (SES) and urbanization degree per postcode area was obtained from Statistics Netherlands (www.cbs.nl).

In total, 9948 (PIENTER-1) and 7904 (PIENTER-2) individuals provided a serum sample. However, for practical and budgetary reasons, a selection of the available sera was tested for IgG antibodies against *Toxocara* and *Ascaris*. In PIENTER-1, a total of 1159 sera were tested for both *Toxocara* and *Ascaris*. In PIENTER-2, 3683 and 3675 sera were tested for *Toxocara* and *Ascaris*, respectively. As mentioned before, the PIENTER studies were meant to evaluate the national immunization program, meaning that the priority was to test the collected sera for a number of vaccine-preventable diseases. Therefore, antibodies for other pathogens like *Toxocara* and *Ascaris* could be searched for only in those sera that had sufficient material to be analysed further. This means that mainly serum samples from (young) children were underrepresented for *Toxocara* and *Ascaris* testing, as they contained relatively smaller quantities of serum than those from adults. Departures of our sample from the underlying population (due to non-random selection, among others) as regard to the variables age, gender, ethnicity and degree of urbanization were accounted for in the analysis using sampling weights (see "Data analysis" section).

Serological analysis

Anti-*Toxocara* and anti-*Ascaris* IgG antibodies in the collected sera were detected using an enzyme-linked immunosorbent assay (ELISA) and the excretory/secretory (E/S) antigen derived from cultivated *T. canis* and *A. suum* larvae as previously described (Pinelli et al. 2009, 2011). Medium binding ELISA microtiter plates (Nunc, Roskilde, Denmark) were used for the *Toxocara* ELISA and high binding plates (Greiner, Frickenhausen, Germany) for the *Ascaris* ELISA. The antibody optical density (OD) units of the tested and reference (cut-off) sera were used to calculate a ratio, with a serum having a ratio ≥ 1.0 being considered positive. The cut-off value was defined as the mean absorbance of 20 serum samples from healthy blood donors plus three times the standard deviation (Pinelli et al. 2009, 2011). The methods, antigens and controls have not been altered between the two serosurveys.

Data analysis

Seroprevalence rates for *Toxocara* and *Ascaris* were estimated either for 1995/1996 (PIENTER-1) or 2005/2006 (PIENTER-2). Generalized linear models with gamma family and log link were used to identify factors associated with increased or decreased OD ratio units for *Toxocara* and *Ascaris* in the tested sera, since these were continuous, positive and right-skew outcome variables with constant variance on the log scale. Models were built in stepwise fashion; 21 (PIENTER-1) and 52 (PIENTER-2) variables were tested for association with the outcome (Tables 5 and 6). After univariate selection ($p < 0.10$) of candidate predictors to be assessed multivariately, non-significant ($p > 0.05$) variables were dropped one by one from the multivariable models after having evaluated each partial effect; variables causing a change of >10 % in the coefficients of the other covariates were retained in the models. Age group, gender, ethnicity, urbanization degree, SES group and education level (see Tables 1, 2, 3 and 4 for categorization of these variables) were always included in the models to account for potential confounding effects. Biologically plausible interactions between independent variables were also assessed. All analyses accounted for the survey design, including the geographical regions as strata, the municipalities as clusters (principal sampling units) and a weighting adjustment for age, gender, ethnicity and urbanization degree to the corresponding population from which the samples were drawn in order to account for deviations of the sample distribution from the general population in the Netherlands. Model residuals were inspected to confirm absence of any remaining structure not accounted for by the models. Statistical analyses were performed using STATA 13 (StataCorp, College Station, USA).

Results

Seroprevalence

Overall *Toxocara* seroprevalence was estimated at 10.7 % (95 % confidence interval [95 % CI] 8.9–12.8 %) in PIENTER-1 and 8.0 % (95 % CI 6.7–9.5 %) in PIENTER-2. For *Ascaris*, seroprevalence was 30.4 % (95 % CI 27.7–33.3 %) in PIENTER-1 and 41.6 % (95 % CI 39.6–43.5 %) in PIENTER-2. Looking at the differences in these seroprevalence rates revealed that while *Toxocara* seroprevalence had decreased significantly from PIENTER-1 to PIENTER-2 ($z = -2.67$, $p = 0.008$), that of *Ascaris* was significantly higher in the second serosurvey compared to the first one ($z = 6.37$, $p < 0.0001$). In general, seroprevalence of both *Toxocara* and *Ascaris* rose with increasing age (Figs. 1 and 2) and decreased with increasing education level (Tables 1, 2, 3 and 4). *Toxocara* seroprevalence was higher in males and in non-autochthonous Dutch individuals, particularly in first-generation migrants from Surinam/Netherlands Antilles (Figs. 1, 2 and 3; Tables 1 and 2). Also *Ascaris* seroprevalence was higher in non-autochthonous Dutch individuals, particularly those of non-Western origin (Fig. 3, Tables 3 and 4).

Factors associated with anti-*Toxocara* IgG levels

Factors independently associated with increased OD ratio units in the final multivariable model for *Toxocara* were increasing age, male gender, having gardened and/or had contact with soil and/or sand (including that of sandpit playgrounds) with bare hands in the last 12 months and having owned cattle, pigs or cats in the last 5 years. These factors were significant in both serosurveys (Tables 1 and 2). In PIENTER-2, other factors associated with increased anti-*Toxocara* OD ratio units were increasing monthly income, having hay fever and being a first-generation migrant from Surinam/Netherlands Antilles or from other non-Western countries (other than Morocco and Turkey) as compared to being autochthonous Dutch (Table 2). Conversely, a very high education (i.e. from university-level institutions) and living in an area with an intermediate degree of urbanization (PIENTER-1) or in moderately urbanized areas (PIENTER-

Table 1 Factors associated with increased or decreased OD ratio units for *Toxocara* in the first nationwide serosurvey (PIENTER-1) in the Netherlands, 1995/1996

Factor	*N* (%)	% Adjusted seroprevalence (95 % CI)	Adjusted mean OD ratio (SE)	Adjusted exponentiated β-coefficient (95 % CI)
Age group (years)				
≤5	64 (5.5)	1.6 (0.0–3.9)	0.06 (0.02)	Reference
6–15	182 (15.7)	6.0 (0.2–11.8)	0.20 (0.06)	2.91 (1.01–8.42)*
16–35	276 (23.8)	11.6 (7.6–15.5)	0.35 (0.04)	5.23 (2.62–10.41)***
35–55	313 (27.0)	11.3 (5.6–17.0)	0.47 (0.10)	6.95 (3.05–15.81)***
56–70	220 (19.0)	18.0 (13.3–22.7)	0.55 (0.05)	8.16 (3.41–19.51)***
≥71	104 (9.0)	15.5 (7.5–23.4)	0.54 (0.09)	8.08 (4.73–13.82)***
Gender				
Female	596 (51.4)	6.7 (4.2–9.1)	0.28 (0.03)	Reference
Male	563 (48.6)	14.4 (11.8–17.0)	0.43 (0.05)	1.56 (1.32–1.84)***
Ethnicity				
Autochthonous Dutch[a]	1057 (91.2)	10.2 (8.1–12.4)	0.35 (0.04)	Reference
Other Western country[b]	40 (3.6)	16.7 (9.2–24.2)	0.65 (0.17)	1.88 (0.95–3.73)
Non-Western country	24 (2.1)	20.2 (0.0–42.0)	0.41 (0.22)	1.19 (0.32–4.43)
Others/unknown	38 (3.3)	11.8 (7.1–16.5)	0.47 (0.11)	1.36 (0.85–2.19)
Urbanization degree[c]				
Urban area	147 (12.7)	13.6 (12.9–14.3)	0.48 (0.05)	Reference
Intermediate area	516 (44.5)	9.1 (7.8–10.4)	0.31 (0.02)	0.63 (0.46–0.86)**
Rural area	496 (42.8)	11.5 (7.4–15.6)	0.38 (0.08)	0.80 (0.46–1.37)
Socio-economic status[d]				
High	611 (52.7)	9.0 (6.3–11.7)	0.32 (0.05)	Reference
Intermediate	338 (29.2)	14.1 (11.2–17.0)	0.44 (0.08)	1.36 (0.74–2.51)
Low	210 (18.1)	11.2 (6.1–16.3)	0.38 (0.07)	1.18 (0.79–1.79)
Education level[e]				
None	156 (13.5)	10.8 (6.5–15.1)	0.37 (0.08)	Reference
Low	350 (30.2)	13.9 (9.7–18.2)	0.43 (0.04)	1.15 (0.81–1.63)
Intermediate	188 (16.2)	8.0 (4.0–12.1)	0.28 (0.05)	0.76 (0.51–1.13)
High	157 (13.5)	6.0 (3.6–8.5)	0.25 (0.03)	0.67 (0.42–1.07)
Very high	24 (2.1)	5.7 (0.0–16.4)	0.11 (0.04)	0.29 (0.13–0.67)**
Unknown	284 (24.5)	17.2 (5.9–28.4)	0.62 (0.12)	1.69 (1.14–2.48)*
Gardened/handled soil with bare hands (past 12 months)				
No	291 (25.1)	9.7 (6.0–13.3)	0.28 (0.04)	Reference
Yes	855 (73.8)	11.0 (9.2–12.7)	0.39 (0.04)	1.39 (1.04–1.85)*
Unknown	13 (1.1)	14.5 (0.0–36.0)	0.33 (0.15)	1.20 (0.37–3.88)
Owned cattle (past 5 years)				
No	1095 (94.5)	10.3 (8.4–12.1)	0.34 (0.03)	Reference
Yes	64 (5.5)	16.0 (9.8–22.1)	0.53 (0.10)	1.55 (1.20–2.01)**
Owned pigs (past 5 years)				
No	1139 (98.3)	10.3 (8.8–11.9)	0.35 (0.03)	Reference
Yes	20 (1.7)	22.7 (0.0–46.7)	0.68 (0.25)	1.94 (1.00–3.79)*
Owned cats (past 5 years)				
No	751 (64.8)	8.8 (7.1–10.5)	0.31 (0.04)	Reference
Yes	408 (35.2)	14.0 (10.1–18.0)	0.44 (0.05)	1.45 (1.20–1.75)**

Estimates are adjusted for all the variables shown in this table

OD optical density of the tested sera, *SE* standard error of the mean, 95 % CI, 95 % confidence interval

[a] Defined as being born in the Netherlands and having both parents being born in the Netherlands as well

[b] Any Western country other than the Netherlands in Europe, North America, Australia and New Zealand

[c] Expressed as addresses/km^2 per postcode: urban area >2000 addresses/km^2, intermediate area 500–2000 addresses/km^2 and rural area <500 addresses/km^2

[d] Expressed as a normalized score ranging from −4 to +4 based on income, employment and educational level per postcode area. Categorized according to tertiles of the socio-economic status score distribution in the whole of the Netherlands

[e] None = no education; low = primary, lower vocational or lower secondary education; intermediate = intermediate vocational or intermediate secondary education; high = higher secondary or higher vocational education; very high = university education

*p value <0.05, **p value ≤0.01, ***p value ≤0.001

Table 2 Factors associated with increased or decreased OD ratio units for *Toxocara* in the second nationwide serosurvey (PIENTER-2) in the Netherlands, 2006/2007

Factor	*N* (%)	% Adjusted seroprevalence (95 % CI)	Adjusted mean OD ratio (SE)	Adjusted exponentiated β-coefficient (95 % CI)
Age group (years)				
≤5	428 (11.6)	2.9 (0.8–4.9)	0.20 (0.02)	Reference
6–15	635 (17.2)	4.9 (3.2–6.6)	0.25 (0.02)	1.25 (1.01–1.55)*
16–35	825 (22.4)	5.6 (3.9–7.4)	0.27 (0.01)	1.38 (1.14–1.68)**
35–55	838 (22.8)	7.8 (6.0–9.7)	0.33 (0.02)	1.69 (1.40–2.04)***
56–70	663 (18.0)	12.9 (9.7–16.1)	0.49 (0.03)	2.49 (1.95–3.19)***
≥71	294 (8.0)	17.5 (13.2–21.7)	0.59 (0.06)	3.01 (2.49–3.64)***
Gender				
Female	1984 (53.9)	6.6 (5.4–7.7)	0.31 (0.01)	Reference
Male	1699 (46.1)	9.5 (7.9–11.0)	0.37 (0.02)	1.20 (1.07–1.34)**
Ethnicity				
Autochthonous Dutch[a]	2978 (80.9)	7.5 (6.3–8.7)	0.32 (0.01)	Reference
First gen. other Western country[b]	74 (2.0)	13.1 (5.3–21.0)	0.35 (0.06)	1.11 (0.81–1.52)
Second gen. other Western country[b]	127 (3.4)	6.4 (1.5–11.4)	0.34 (0.04)	1.07 (0.85–1.35)
First gen. Morocco/Turkey	118 (3.2)	6.1 (0.0–14.5)	0.32 (0.09)	1.00 (0.57–1.74)
Second gen. Morocco/Turkey	57 (1.6)	5.2 (0.0–13.6)	0.31 (0.08)	0.97 (0.56–1.68)
First gen. Surinam/Netherlands Antilles	100 (2.7)	32.0 (22.0–42.0)	1.14 (0.17)	3.59 (2.62–4.92)***
Second gen. Surinam/Netherlands Antilles	52 (1.4)	7.2 (0.0–14.6)	0.35 (0.08)	1.08 (0.66–1.77)
First gen. other non-Western country[c]	117 (3.2)	10.3 (4.3–16.3)	0.47 (0.08)	1.48 (1.05–2.08)*
Second gen. other non-Western country[c]	60 (1.6)	12.6 (0.9–24.3)	0.48 (0.11)	1.52 (0.93–2.48)
Urbanization degree[d]				
Highly urbanized area	778 (21.1)	8.0 (4.8–11.1)	0.34 (0.03)	Reference
Urbanized area	1407 (38.2)	6.6 (5.4–7.8)	0.31 (0.02)	0.91 (0.74–1.12)
Moderately urbanized area	126 (3.4)	5.2 (3.5–7.0)	0.26 (0.02)	0.76 (0.60–0.96)*
Lowly urbanized area	637 (17.3)	9.4 (6.1–12.6)	0.33 (0.03)	0.96 (0.74–1.24)
Rural area	735 (20.0)	10.8 (7.0–14.6)	0.42 (0.04)	1.24 (0.96–1.60)
Socio-economic status[e]				
High	1031 (28.0)	9.0 (7.3–10.7)	0.37 (0.02)	Reference
Intermediate	1144 (31.1)	7.3 (5.5–9.0)	0.33 (0.02)	0.89 (0.76–1.04)
Low	1508 (40.9)	7.9 (5.8–10.0)	0.32 (0.02)	0.87 (0.76–1.00)*

Table 2 (continued)

Factor	*N* (%)	% Adjusted seroprevalence (95 % CI)	Adjusted mean OD ratio (SE)	Adjusted exponentiated β-coefficient (95 % CI)
Education level[f]				
None	133 (3.6)	11.7 (5.4–17.9)	0.31 (0.04)	Reference
Low	869 (23.6)	11.2 (8.4–14.0)	0.40 (0.03)	1.28 (0.98–1.67)
Intermediate	1408 (38.2)	7.2 (5.8–8.5)	0.32 (0.01)	1.02 (0.79–1.31)
High	945 (25.7)	7.1 (5.3–8.8)	0.32 (0.02)	1.03 (0.77–1.37)
Very high	263 (7.1)	3.1 (1.5–4.6)	0.24 (0.02)	0.76 (0.56–1.04)
Unknown	65 (1.8)	16.0 (5.1–26.9)	0.56 (0.18)	1.79 (0.85–3.76)
Net monthly income (€)				
<1150	549 (14.9)	8.1 (6.2–10.0)	0.29 (0.02)	Reference
1151–1750	748 (20.3)	7.9 (6.0–9.9)	0.36 (0.03)	1.25 (1.04–1.49)*
1751–3050	1039 (28.2)	8.2 (6.3–10.2)	0.33 (0.02)	1.14 (0.97–1.35)
>3051	574 (15.6)	9.3 (7.1–11.5)	0.39 (0.03)	1.35 (1.11–1.64)**
Unknown	773 (21.0)	6.9 (4.6–9.2)	0.32 (0.02)	1.10 (0.94–1.28)
Gardened/handled soil/sand with bare hands (past 12 months)				
No	1061 (28.8)	6.6 (4.6–8.6)	0.30 (0.02)	Reference
Yes	2622 (71.2)	8.5 (7.2–9.9)	0.35 (0.02)	1.17 (1.02–1.34)*
Suffering from hay fever				
No	3203 (87.0)	7.8 (6.9–8.8)	0.33 (0.01)	Reference
Yes	480 (13.0)	9.3 (6.1–12.5)	0.39 (0.03)	1.20 (1.07–1.35)**
Owned cattle (past 5 years)				
No	3600 (97.8)	7.7 (6.6–8.8)	0.33 (0.01)	Reference
Yes	83 (2.2)	20.5 (10.2–30.9)	0.63 (0.09)	1.93 (1.46–2.55)***
Owned pigs (past 5 years)				
No	3659 (99.4)	8.0 (7.0–9.0)	0.33 (0.01)	Reference
Yes	24 (0.6)	10.6 (2.8–18.4)	0.66 (0.12)	1.98 (1.38–2.84)
Owned/being exposed to cats (past 5 years)				
No	1546 (42.0)	7.5 (6.1–8.9)	0.32 (0.01)	Reference
Yes	2137 (58.0)	8.4 (7.2–9.6)	0.35 (0.01)	1.12 (1.01–1.23)

Estimates are adjusted for all the variables shown in this table

OD optical density of the tested sera, *SE* standard error of the mean, *95 % CI* 95 % confidence interval

[a] Defined as being born in the Netherlands and having both parents being born in the Netherlands as well

[b] Any Western country other than the Netherlands in Europe, North America, Australia and New Zealand

[c] Any non-Western country other than Morocco, Turkey, Surinam and Netherlands Antilles

[d] Expressed as addresses/km^2 per postcode: highly urbanized area >2000 addresses/km^2, urbanized area 1500–2000 addresses/km^2, moderately urbanized area 1000–1500 addresses/km^2, lowly urbanized area 500–1000 addresses/km^2 and rural area <500 addresses/km^2

[e] Expressed as a normalized score ranging from −4 to +4 based on income, employment and educational level per postcode area. Categorized according to tertiles of the socio-economic status score distribution in the whole of the Netherlands

[f] None = no education; low = primary, lower vocational or lower secondary education; intermediate = intermediate vocational or intermediate secondary education; high = higher secondary or higher vocational education; very high = university education

*p value <0.05, **p value ≤0.01, ***p value ≤0.001

Table 3 Factors associated with increased or decreased OD ratio units for *Ascaris* in the first nationwide serosurvey (PIENTER-1) in the Netherlands, 1995/1996

Factor	*N* (%)	% Adjusted seroprevalence (95 % CI)	Adjusted mean OD ratio (SE)	Adjusted exponentiated β-coefficient (95 % CI)
Age group (years)				
≤5	64 (5.5)	13.5 (1.4–25.6)	0.46 (0.10)	Reference
6–15	182 (15.7)	13.7 (8.1–19.3)	0.52 (0.06)	1.11 (0.70–1.77)
16–35	276 (23.8)	22.9 (17.1–28.6)	0.64 (0.05)	1.38 (0.75–2.53)
35–55	313 (27.0)	38.9 (34.6–43.3)	0.98 (0.05)	2.11 (1.29–3.46)**
56–70	220 (19.0)	40.0 (31.7–48.3)	1.05 (0.09)	2.26 (1.24–4.14)*
≥71	104 (9.0)	42.0 (24.6–59.4)	1.11 (0.14)	2.41 (1.20–4.85)*
Gender				
Female	596 (51.4)	29.9 (24.6–35.2)	0.83 (0.07)	Reference
Male	563 (48.6)	30.9 (24.8–37.0)	0.82 (0.06)	0.99 (0.74–1.34)
Ethnicity				
Autochthonous Dutch[a]	1057 (91.2)	30.0 (27.0–33.1)	0.81 (0.04)	Reference
Other Western country[b]	40 (3.6)	38.2 (34.0–42.4)	1.03 (0.12)	1.28 (0.99–1.64)
Non-Western country	24 (2.1)	43.0 (2.4–83.6)	1.11 (0.28)	1.37 (0.79–2.40)
Others/unknown	38 (3.3)	25.4 (11.1–39.7)	1.01 (0.18)	1.25 (0.80–1.95)
Urbanization degree[c]				
Urban area	147 (12.7)	27.4 (24.7–30.1)	0.75 (0.02)	Reference
Intermediate area	516 (44.5)	30.4 (25.1–35.8)	0.83 (0.08)	1.10 (0.88–1.37)
Rural area	496 (42.8)	31.3 (26.2–36.4)	0.85 (0.04)	1.13 (0.96–1.31)
Socio-economic status[d]				
High	611 (52.7)	34.9 (29.2–40.7)	0.90 (0.06)	Reference
Intermediate	338 (29.2)	25.6 (16.1–35.1)	0.76 (0.10)	0.84 (0.55–1.28)
Low	210 (18.1)	24.4 (15.5–35.3)	0.72 (0.07)	0.80 (0.59–1.09)
Education level[e]				
None	156 (13.5)	33.9 (23.2–44.7)	0.85 (0.06)	Reference
Low	350 (30.2)	28.9 (21.3–36.5)	0.83 (0.08)	0.97 (0.84–1.12)
Intermediate	188 (16.2)	33.6 (22.3–44.8)	0.83 (0.07)	0.97 (0.70–1.34)
High	157 (13.5)	32.1 (24.2–40.0)	0.95 (0.15)	1.11 (0.75–1.65)
Very high	24 (2.1)	23.3 (2.6–44.1)	0.78 (0.33)	0.91 (0.32–2.61)
Unknown	284 (24.5)	25.6 (14.1–37.1)	0.66 (0.09)	0.78 (0.50–1.21)
Owned pigs (past 5 years)				
No	1139 (98.3)	29.7 (26.6–32.9)	0.81 (0.04)	Reference
Yes	20 (1.7)	65.4 (50.0–80.8)	1.55 (0.25)	1.91 (1.36–2.72)**
Owned rabbits (past 5 years)				
No	923 (79.6)	30.8 (27.4–34.2)	0.85 (0.04)	Reference
Yes	236 (20.4)	28.9 (25.9–32.0)	0.74 (0.04)	0.87 (0.28–0.81)**

Estimates are adjusted for all the variables shown in this table

OD optical density of the tested sera, *SE* standard error of the mean, *95 % CI* 95 % confidence interval

[a] Defined as being born in the Netherlands and having both parents being born in the Netherlands as well

[b] Any Western country other than the Netherlands in Europe, North America, Australia and New Zealand

[c] Expressed as addresses/km^2 per postcode: urban area >2000 addresses/km^2, intermediate area 500–2000 addresses/km^2 and rural area <500 addresses/km^2

[d] Expressed as a normalized score ranging from −4 to +4 based on income, employment and educational level per postcode area. Categorized according to tertiles of the socio-economic status score distribution in the whole of the Netherlands

[e] None = no education; low = primary, lower vocational or lower secondary education; intermediate = intermediate vocational or intermediate secondary education; high = higher secondary or higher vocational education; very high = university education

**p* value <0.05, ***p* value ≤0.01, ****p* value ≤0.001

2) were independently associated with decreased OD ratio units for *Toxocara* (Tables 1 and 2).

Factors associated with anti-*Ascaris* IgG levels

In both PIENTER-1 and PIENTER-2, increasing age and having owned pigs in the last 5 years were independently associated with increased OD ratio units in the final multivariable model for *Ascaris* (Tables 3 and 4). A significant interaction was found between geophagia and (preschool) age, with ≤5-year-old children displaying geophagia having increased anti-*Ascaris* antibodies as compared to non-geophagic children (Table 4). Other factors associated with increased anti-*Ascaris* antibodies in PIENTER-2 were being

Table 4 Factors associated with increased or decreased OD ratio units for *Ascaris* in the second nationwide serosurvey (PIENTER-2) in the Netherlands, 2006/2007

Factor	*N* (%)	% Adjusted seroprevalence (95 % CI)	Adjusted mean OD ratio (SE)	Adjusted exponentiated β-coefficient (95 % CI)
Age group (years)				
≤5, displaying no geophagia	332 (9.0)	14.4 (10.6–18.1)	0.55 (0.04)	Reference
≤5, displaying geophagia	93 (2.5)	24.5 (15.5–33.5)	0.73 (0.08)	1.32 (1.05–1.68)*
6–15	633 (17.2)	21.6 (17.8–25.4)	0.72 (0.04)	1.29 (1.11–1.52)***
16–35	823 (22.4)	38.2 (35.1–41.2)	1.18 (0.05)	2.14 (1.84–2.48)***
35–55	839 (22.9)	47.6 (44.5–50.7)	1.40 (0.05)	2.53 (2.20–2.91)***
56–70	662 (18.0)	55.4 (51.4–59.5)	1.59 (0.06)	2.88 (2.50–3.32)***
≥71	293 (8.0)	64.0 (57.9–70.1)	1.86 (0.12)	3.37 (2.84–4.00)***
Gender				
Female	1980 (53.9)	42.3 (40.4–44.1)	1.27 (0.03)	Reference
Male	1695 (46.1)	40.9 (38.1–43.6)	1.21 (0.03)	0.95 (0.88–1.02)
Ethnicity				
Autochthonous Dutch[a]	2972 (80.9)	39.7 (37.9–41.4)	1.19 (0.02)	Reference
First gen. other Western country[b]	74 (2.0)	47.2 (39.8–54.6)	1.63 (0.19)	1.38 (1.09–1.74)**
Second gen. other Western country[b]	126 (3.4)	38.8 (31.4–46.2)	1.22 (0.12)	1.03 (0.84–1.26)
First gen. Morocco/Turkey	118 (3.2)	58.1 (50.8–65.4)	1.65 (0.09)	1.39 (1.24–1.57)***
Second gen. Morocco/Turkey	57 (1.6)	39.7 (28.3–51.1)	1.25 (0.17)	1.06 (0.79–1.40)
First gen. Surinam/Netherlands Antilles	99 (2.7)	56.1 (48.9–63.3)	1.51 (0.11)	1.28 (1.10–1.48)**
Second gen. Surinam/Netherlands Antilles	52 (1.4)	60.1 (48.4–71.7)	1.72 (0.17)	1.45 (1.18–1.78)**
First gen. other non-Western country[c]	117 (3.2)	58.8 (49.3–68.4)	1.78 (0.16)	1.50 (1.25–1.80)***
Second gen. other non-Western country[c]	60 (1.6)	62.9 (49.9–76.0)	1.87 (0.30)	1.58 (1.14–2.17)**
Urbanization degree[d]				
Highly urbanized area	779 (21.2)	43.2 (40.1–46.3)	1.30 (0.03)	Reference
Urbanized area	1402 (38.2)	40.6 (38.5–42.8)	1.23 (0.03)	0.95 (0.89–1.03)
Moderately urbanized area	126 (3.4)	48.8 (45.0–52.5)	1.28 (0.03)	0.99 (0.92–1.07)
Lowly urbanized area	636 (17.3)	42.6 (36.4–48.7)	1.26 (0.06)	0.97 (0.87–1.09)
Rural area	732 (19.9)	39.4 (34.6–44.3)	1.16 (0.06)	0.89 (0.80–1.00)*
Socio-economic status[e]				
High	1031 (28.0)	42.7 (38.7–46.8)	1.30 (0.04)	Reference
Intermediate	1142 (31.1)	39.9 (36.4–43.5)	1.20 (0.03)	0.92 (0.86–1.00)
Low	1502 (40.9)	41.9 (38.5–45.2)	1.22 (0.04)	0.95 (0.87–1.02)
Education level[f]				
None	132 (3.6)	49.9 (43.2–56.5)	1.40 (0.08)	Reference
Low	869 (23.7)	45.8 (42.9–48.7)	1.29 (0.04)	0.93 (0.83–1.04)
Intermediate	1408 (38.2)	43.3 (40.6–46.0)	1.30 (0.03)	0.94 (0.84–1.05)
High	943 (25.7)	35.1 (32.2–38.1)	1.11 (0.03)	0.80 (0.71–0.89)***
Very high	263 (7.2)	39.8 (34.3–45.3)	1.07 (0.07)	0.77 (0.64–0.91)**
Unknown	65 (1.8)	45.6 (35.0–56.1)	1.59 (0.21)	1.14 (0.87–1.49)
Net monthly income (€)				
<1150	549 (14.9)	42.8 (37.3–48.2)	1.23 (0.07)	Reference
1151–1750	745 (20.3)	43.3 (40.6–46.0)	1.28 (0.04)	1.04 (0.91–1.20)
1751–3050	1036 (28.2)	39.4 (36.8–42.0)	1.22 (0.04)	0.97 (0.87–1.15)
>3051	573 (15.6)	42.5 (38.3–46.7)	1.30 (0.06)	1.06 (0.90–1.24)
Unknown	772 (21.0)	41.4 (37.9–44.9)	1.19 (0.05)	0.97 (0.85–1.11)
Owned pigs (past 5 years)				
No	3651 (99.4)	41.5 (40.0–43.0)	1.23 (0.02)	Reference
Yes	24 (0.6)	55.9 (37.9–73.9)	1.93 (0.07)	1.57 (1.01–2.42)*
Owned poultry (past 5 years)				
No	3507 (95.4)	41.9 (40.4–43.4)	1.25 (0.02)	Reference
Yes	168 (4.6)	33.1 (25.6–40.5)	1.01 (0.07)	0.81 (0.70–0.93)**

Estimates are adjusted for all the variables shown in this table

OD optical density of the tested sera, *SE* standard error of the mean, *95 % CI* 95 % confidence interval

[a] Defined as being born in the Netherlands and having both parents being born in the Netherlands as well

[b] Any Western country other than the Netherlands in Europe, North America, Australia and New Zealand

[c] Any non-Western country other than Morocco, Turkey, Surinam and Netherlands Antilles

[d] Expressed as addresses/km^2 per postcode: highly urbanized area >2000 addresses/km^2, urbanized area 1500–2000 addresses/km^2, moderately urbanized area 1000–1500 addresses/km^2, lowly urbanized area 500–1000 addresses/km^2 and rural area <500 addresses/km^2

[e] Expressed as a normalized score ranging from −4 to +4 based on income, employment and educational level per postcode area. Categorized according to tertiles of the socio-economic status score distribution in the whole of the Netherlands

[f] None = no education; low = primary, lower vocational or lower secondary education; intermediate = intermediate vocational or intermediate secondary education; high = higher secondary or higher vocational education; very high = university education

**p* value <0.05, ***p* value ≤0.01, ****p* value ≤0.001

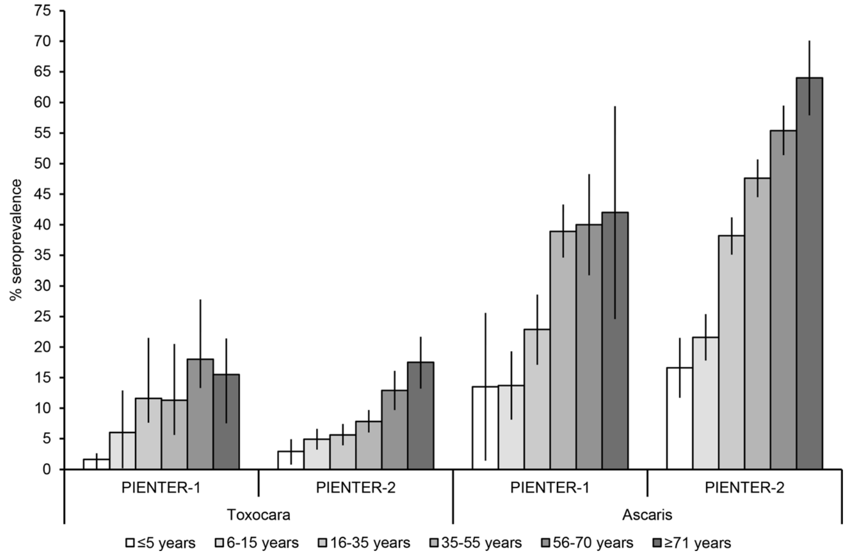

Fig. 1 *Toxocara* and *Ascaris* seroprevalence estimates per age group in the first (PIENTER-1) and in the second (PIENTER-2) nationwide serosurvey in the Netherlands. *Error bars* represent 95 % confidence intervals. *Toxocara* and *Ascaris* prevalence estimates from PIENTER-1 and PIENTER-2 are adjusted for the variables shown in Tables 1, 2, 3 and 4, respectively

a first-generation migrant from either Morocco/Turkey or from a Western country (in Europe, North America, Australia and New Zealand) other than the Netherlands or being a first- or second-generation migrant from either Surinam/Netherlands Antilles or from other non-Western countries, vs. being an autochthonous Dutch. Factors independently associated with decreased OD ratio units for *Ascaris* were increasing education level and having owned rabbits or poultry in the last 5 years (Tables 3 and 4).

Discussion

While the seroprevalence of *Toxocara* in the general Dutch population was found to decrease from 10.7 % in 1995/1996 to 8.0 % in 2006/2007, the one of *Ascaris* increased from 30.4 to 41.6 % during the same period. These trends agree with our previous findings on *Toxocara* and *Ascaris* seropositivity among patients suspected of VLM/OLM during 1998–2009 in the Netherlands (Pinelli et al. 2011), as in these patients, *Toxocara* seropositivity decreased significantly over time, whereas *Ascaris* seropositivity remained unchanged. Campaigns promoting regular deworming of dogs and cats have existed for many years in the Netherlands (Overgaauw and Boersema 1996). Although this might explain the observed decrease in *Toxocara* seroprevalence, it is difficult to ignore that regular deworming of cats is infrequent (Overgaauw et al. 2009) and that *Toxocara* prevalence in dogs has remained almost unchanged (at relatively low levels of ~5 %) over the last two decades in the Netherlands (Nijsse et al. 2015b). A recent modelling paper (Nijsse et al. 2015a) also suggested that cats, rather than dogs, are responsible for most of the environmental contamination with *Toxocara* eggs, accounting for 46 % of the overall *Toxocara* egg output of >6-

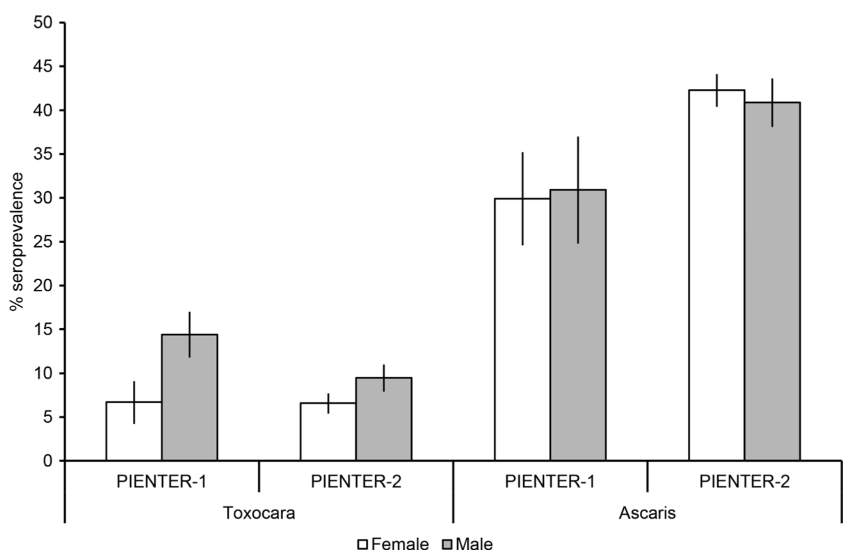

Fig. 2 *Toxocara* and *Ascaris* seroprevalence estimates per gender in the first (PIENTER-1) and in the second (PIENTER-2) nationwide serosurvey in the Netherlands. *Error bars* represent 95 % confidence intervals. *Toxocara* and *Ascaris* prevalence estimates from PIENTER-1 and PIENTER-2 are adjusted for the variables shown in Tables 1, 2, 3 and 4, respectively

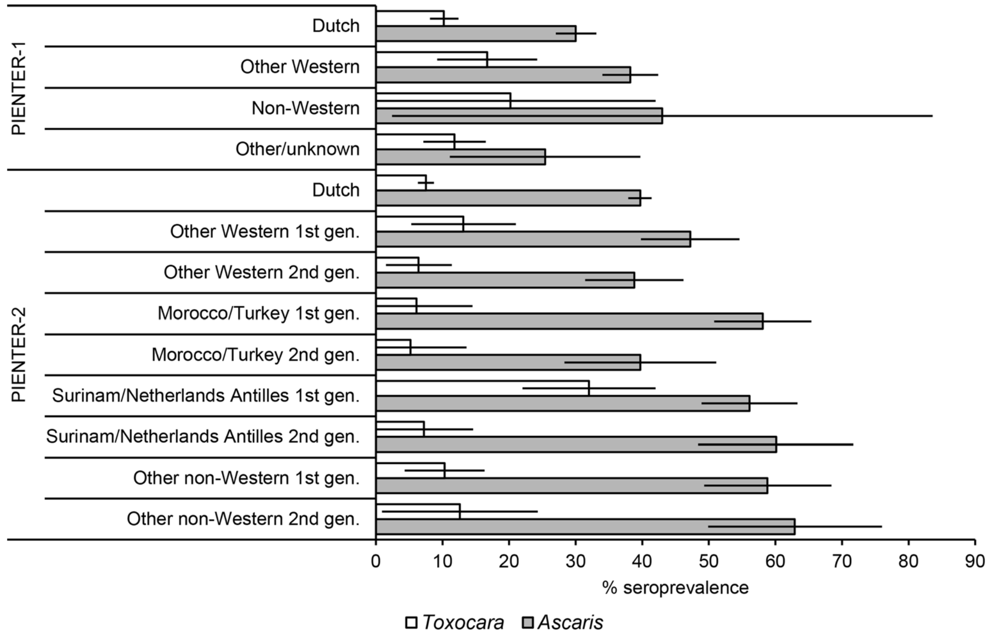

Fig. 3 *Toxocara* and *Ascaris* seroprevalence per ethnic group in the first (PIENTER-1) and in the second (PIENTER-2) nationwide serosurvey in the Netherlands. *Error bars* represent 95 % confidence intervals. *Toxocara* and *Ascaris* prevalence estimates from PIENTER-1 and PIENTER-2 are adjusted for the variables shown in Tables 1, 2, 3 and 4, respectively

month-old hosts in the Netherlands, followed by dogs (39 %) and foxes (15 %). The same study also reported that *Toxocara* egg output in urban areas is dominated by free-ranging cats (81 %), as the Netherlands is a country free of stray dogs. Moreover, simulated intervention scenarios indicated that the currently advocated four-times-a-year deworming advice for adult dogs would have little impact on environmental contamination with *Toxocara* eggs unless its compliance is very high (>90 %) (Nijsse et al. 2015a), which is not realistically enforceable. Altogether, these findings suggest that nowadays dogs might not be the most problematic source of human toxocariasis in a country like the Netherlands, so deworming is unlikely to explain the observed decrease in *Toxocara* seroprevalence. Rather, such decrease is likely to reflect the interplay of several factors entailing hygiene enhancements between 1995/1996 and 2006/2007, e.g. more frequent enforcement of clean-up/disposal of dog faeces as well as prohibition of dogs being kept off-leash in most places, establishment of dedicated (confined) walking areas for dogs in urban areas, increased use of commercial pet food as opposed to (raw) kitchen scraps, increased practice of covering sandpit playgrounds when not in use, periodic replacement of sand in public sandpits, etc.

The use of larval ES antigens is the most common approach for serodiagnosis of *Toxocara* and *Ascaris* infections in humans (Smith et al. 2009; van Knapen et al. 1992). It is worth mentioning that due to cross-reactivity, the *Toxocara* ELISA does not differentiate between *T. canis* and *T. cati* and the *Ascaris* ELISA does not differentiate between *A. suum* and *A. lumbricoides*. Moreover, we cannot rule out that cross-reactivity with other helminths like *Toxocara leonina* might occur, as this has not yet been assessed. However, autochthonous *A. lumbricoides* infections are virtually absent in the Netherlands as indicated by at least two large population-based studies on enteropathogens in stool samples screened for different parasites and in which no helminth eggs were found (de Wit et al. 2001a, b). Indeed, *A. lumbricoides* has a worldwide distribution, but it occurs primarily in developing countries where conditions of poor sanitization exist, whereas it is extremely rare in developed countries (Bethony et al. 2006; Umetsu et al. 2014). Moreover, people infected with *A. lumbricoides* do not always develop circulating antibodies, as these are not usually elicited by the presence of adult worms in the intestine but rather by the extra-intestinal larval migrations in proportion to the number of migrating larvae. Therefore, small numbers of migrating larvae and short-lasting migrations may not suffice to prompt a detectable humoural immune response (Haswell-Elkins et al. 1992). Importantly, the Netherlands is one of the largest pig producers in Europe, and the farm-level prevalence of *A. suum* in Dutch swine farms is as high as 50 % in free-range farms, 73 % in organic farms and 11 % in conventional farms, with fattening pigs within conventional farms showing the highest *A. suum* prevalence (55 %) (Eijck and Borgsteede 2005). Therefore, our results about *Ascaris* more likely refer to *A. suum* than to *A. lumbricoides*. Yet, in both PIENTER-1 and PIENTER-2, *Ascaris* seroprevalence was higher in non-Dutch participants, particularly those from non-Western countries, where *A. lumbricoides* infection might still be frequent.

Although *Toxocara* and *Ascaris* have common antigens that can lead to cross-reactivity (Lozano et al. 2004), this has been reported to be below the detection limit of the respective assays (Pinelli et al. 2009). Moreover, we found different trends in the seroprevalences of *Toxocara* and *Ascaris*, and

Ascaris seroprevalence was much higher than the one of *Toxocara*, making any cross-reactivity unlikely to have affected the results. *Ascaris* seroprevalence was, however, surprisingly high, as it seems rather unclear how the general population is exposed to *A. suum* at a growing rate. Pig manure, which has widely been used as fertilizer for arable lands and compost soil during the 10 years between the two serosurveys, is often contaminated with *A. suum* eggs (van Knapen et al. 1992). Thus, contaminated soil has long been deemed a likely source of human *A. suum* infections in the Netherlands (Pinelli et al. 2009, 2011) and in other European countries (Schneider and Auer 2015), whereas the exposure to *A. suum* through food remains to be investigated. A recent Austrian study examined 4481 sera from patients with suspected VLM during 2012–2014 using immunoblot and found an *A. suum* seropositivity of 13.2 %, which is lower than any *A. suum* seropositivity recorded in the Netherlands. As the Austrian study pointed out, besides differences in pig production and possibly the amount of pig manure contaminated with *A. suum* eggs used as fertilizer, alternative explanations for the observed difference might be the overrepresentation of the urban population with a putative lower risk of infection in the Austrian study, the diagnostic assay used (immunoblot vs. ELISA) and possible reactions with other antigens in the Dutch sera (Schneider and Auer 2015). Therefore, fine tuning of the cut-off to increase specificity are warranted in future studies to further address this matter.

Antibody levels for both *Toxocara* and *Ascaris* increased with age, suggesting continuous exposure to these helminths throughout life. While no significant gender effects were found for *Ascaris*, males showed significantly higher *Toxocara* OD ratio units than females. This has been reported previously (Pinelli et al. 2011) and might be related to traditionally male-oriented behaviours and activities, including the occupation. A study on risk factors for *Toxocara* infections in different occupational groups found that farmers have the highest *Toxocara* seroprevalence (44 %), with free-roaming farm cats and dogs being indicated as the main sources (Deutz et al. 2005). This agrees with our other finding that having owned cattle or pigs in the previous 5 years was associated with increased levels of anti-*Toxocara* antibodies, as these animals are highly unlikely to play a role in the direct transmission of these helminths to humans but rather act as proxies for rural lifestyle. A direct causal relationship may instead be derived from the positive associations we found between cat ownership and increased anti-*Toxocara* antibodies and pig ownership and increased anti-*Ascaris* antibodies.

We found that exposure to *Toxocara* was more likely to occur in people having direct contact with soil, like those gardening with bare hands or playing in a sandpit, and that preschool children exhibiting geophagia have higher levels of anti-*Ascaris* antibodies. This agrees with the positive association between having a high income and increased anti-*Toxocara* antibodies, as wealthy people in the Netherlands are more likely to have houses with gardens. However, the lack of a significant gender effect for *Ascaris* is somewhat suggestive that also food plays a role, as hypothesized previously (Pinelli et al. 2011; Schneider and Auer 2015), and that the association between high education and decreased anti-*Ascaris* or anti-*Toxocara* antibody levels may essentially mirror the effect of (the knowledge of) hygiene measures in addition to occupation. We also found that suffering from hay fever was associated with increased anti-*Toxocara* antibody levels. As mentioned in the introduction, there is evidence indicating that migration of *Toxocara* larvae through the lungs may result in hyper-reactivity of the airways, contributing to the development of allergic manifestations (Pinelli and Aranzamendi 2012). Our findings about the "protective" effects of living in moderately urbanized areas, as well as owning rabbits or poultry, are difficult to grasp and might act as proxies of other (hitherto unknown) factors entailing a lower risk of encountering *Toxocara* and/or *Ascaris*.

One of the most interesting results was the effect of ethnic background on increased levels of anti-*Toxocara* and anti-*Ascaris* antibodies. This concerned specifically the first-generation migrants from Surinam/Netherlands Antilles and from other non-Western countries (excluding Turkey and Morocco) for *Toxocara* and either the first- or second-generation migrants from Surinam/Netherlands Antilles and from other non-Western countries, as well as the first-generation migrants from Turkey or Morocco, for *Ascaris*. Ethnicity as a risk factor for *Toxocara* infection has been reported in the USA for Hispanic children of Puerto Rican descendent (Sharghi et al. 2001). However, it is largely unclear whether these associations are due to specific lifestyle characteristics posing these ethnic groups at increased risk of helminth infections or whether this is due to the high frequency of travel to the countries of origin to visit relatives and friends. Surely, free-ranging dogs and cats are widespread in many non-Western countries, including the Caribbean basin (Georges and Adesiyun 2008; Krecek et al. 2010; Thompson et al. 1986), where *Toxocara* seroprevalence in humans is generally much higher than that in the Netherlands, especially among children (e.g. 40–86 % in Caribbean children vs. 2–6 % in those of the present study) (Baboolal and Rawlins 2002; Kanobana et al. 2013; Lynch et al. 1993; Thompson et al. 1986). This applies to *Ascaris* as well (Lynch et al. 1993), whereas countries with predominantly Muslim population are hardly exposed to pigs (either via food or the environment) given their religious restrictions on the consumption of pork and the very limited pig farming therein. Thus, the significantly higher levels of anti-*Ascaris* antibodies we found among the first-generation migrants from Turkey/Morocco were quite unexpected. However, there are reports of relatively high rates of *A. lumbricoides* infection in these countries (El Guamri et al. 2011; Yentur Doni et al. 2015), so cross-reactivity might be an explanation. It is nonetheless interesting to note that the levels of anti-*Ascaris* antibodies among the second-generation migrants from Turkey/Morocco did not differ significantly from those of the autochthonous Dutch population. This suggests that people who are born and live in a virtually *A. lumbricoides*-free country like the Netherlands and refrain from consuming pork would show similar levels of antibodies against *Ascaris* than those of the native (and largely pork-consuming) Dutch population, thereby supporting the hypothesis of predominant exposure to *Ascaris* through the environment.

In conclusion, we reported different trends for *Toxocara* and *Ascaris* seroprevalences in the Netherlands, possibly reflecting improvements in hygiene management of pets and increased exposure to soil contaminated with pig manure. Future research should focus on the sources of *A. suum* contamination in the environment, not only in the Netherlands but also in other swine-reach regions. Besides identifying several factors associated with increased anti-*Toxocara* and anti-*Ascaris* antibodies, allowing for the identification of specific (age, ethnic, socio-economic, etc.) groups of the general population at high risk of *Toxocara* or *Ascaris* infections, our results suggest that these infections mainly occur through environmental, rather than foodborne, routes, with direct contact with soil and ownership of definitive hosts like cats and pigs being potentially modifiable exposures.

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Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. PIENTER-1 was approved by the Medical Ethical Committee of the Netherlands' Organisation for Applied Scientific Research (TNO) in Leiden. PIENTER-2 has received ethical approval by the Medical Ethics Testing Committee of the Foundation of Therapeutic Evaluation of Medicines (METC-STEG) in Almere (ISRCTN 20164309). All participants and parents/legal caretakers of minors involved in both studies provided written informed consent. No person identifying information was generated in this study.

Conflicts of interest All authors have no conflicts of interest to declare.

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Appendix

Table 5 Variables tested for association with *Toxocara* and *Ascaris* IgG antibodies in the first nationwide serosurvey (PIENTER-1) in the Netherlands, 1995/1996

Variables always retained in the models	*N* (%)
Age group (years)	
≤5	64 (5.5)
6–15	182 (15.7)
16–35	276 (23.8)
35–55	313 (27.0)
56–70	220 (19.0)
≥71	104 (9.0)
Gender	
Female	596 (51.4)
Male	563 (48.6)
Ethnicity	
Autochthonous Dutch[a]	1057 (91.2)
Other Western country[b]	40 (3.6)
Non-Western country	24 (2.1)
Others/unknown	38 (3.3)
Urbanization degree[c]	
Urban area	147 (12.7)
Intermediate area	516 (44.5)
Rural area	496 (42.8)
Socio-economic status[d]	
High	611 (52.7)
Intermediate	338 (29.2)
Low	210 (18.1)
Education level[e]	
None	156 (13.5)
Low	350 (30.2)
Intermediate	188 (16.2)
High	157 (13.5)
Very high	24 (2.1)
Unknown	284 (24.5)
Variables subject to stepwise selection	
Gardened/handled soil with bare hands (past 12 months)	
No	291 (25.1)
Yes	855 (73.8)
Unknown	13 (1.1)
Owned cattle (past 5 years)	
No	1095 (94.5)
Yes	64 (5.5)
Owned pigs (past 5 years)	
No	1139 (98.3)
Yes	20 (1.7)
Owned sheep (past 5 years)	
No	1094 (94.4)
Yes	65 (5.6)
Owned cats (past 5 years)	

Table 5 (continued)

Variables always retained in the models	*N* (%)
No	751 (64.8)
Yes	408 (35.2)
Owned dogs (past 5 years)	
No	742 (64.0)
Yes	417 (36.0)
Owned pets other than dogs and cats (past 5 years)	
No	1056 (91.1)
Yes	103 (8.9)
Owned rabbits (past 5 years)	
No	923 (79.6)
Yes	236 (20.4)
Owned poultry (past 5 years)	
No	1062 (91.6)
Yes	97 (8.4)
Suffering from allergies	
No	1120 (91.6)
Yes	2 (0.2)
Unknown	37 (3.2)
Suffering from asthma	
No	1035 (89.3)
Yes	87 (7.5)
Unknown	37 (3.2)
Playing in a sandpit	
No	1019 (87.9)
Yes	140 (12.1)
Eaten sand	
Never	148 (12.8)
Yes	28 (2.4)
Unknown/not applicable	983 (84.8)
Children in the house attending day care centres	
No	995 (85.9)
Yes	72 (6.2)
Unknown	92 (7.9)
Been abroad for more than 3 months	
No	1036 (89.4)
Yes	123 (10.6)

[a] Defined as being born in the Netherlands and having both parents being born in the Netherlands as well

[b] Any Western country other than the Netherlands in Europe, North America, Australia and New Zealand

[c] Expressed as addresses/km^2 per postcode: urban area >2000 addresses/km^2, intermediate area 500–2000 addresses/km^2 and rural area <500 addresses/km^2

[d] Expressed as a normalized score ranging from −4 to +4 based on income, employment and educational level per postcode area. Categorized according to tertiles of the socio-economic status score distribution in the whole of the Netherlands

[e] None = no education; low = primary, lower vocational or lower secondary education; intermediate = intermediate vocational or intermediate secondary education; high = higher secondary or higher vocational education; very high = university education

Table 6 Variables tested for association with *Toxocara* and *Ascaris* IgG antibodies in the second nationwide serosurvey (PIENTER-2) in the Netherlands, 2006/2007

Variables always retained in the models	*Toxocara* *N* (%)	*Ascaris* *N* (%)
Age group (years)		
≤5	428 (11.6)	425 (11.5)
6–15	635 (17.2)	633 (17.2)
16–35	825 (22.4)	823 (22.4)
35–55	838 (22.8)	839 (22.9)
56–70	663 (18.0)	662 (18.0)
≥71	294 (8.0)	293 (8.0)
Gender		
Female	1984 (53.9)	1980 (53.9)
Male	1699 (46.1)	1695 (46.1)
Ethnicity		
Autochthonous Dutch[a]	2978 (80.9)	2972 (80.9)
First gen. other Western country[b]	74 (2.0)	74 (2.0)
Second gen. other Western country[b]	127 (3.4)	126 (3.4)
First gen. Morocco/Turkey	118 (3.2)	118 (3.2)
Second gen. Morocco/Turkey	57 (1.6)	57 (1.6)
First gen. Surinam/Netherlands Antilles	100 (2.7)	99 (2.7)
Second gen. Surinam/Netherlands Antilles	52 (1.4)	52 (1.4)
First gen. other non-Western country[c]	117 (3.2)	117 (3.2)
Second gen. other non-Western country[c]	60 (1.6)	60 (1.6)
Urbanization degree[d]		
Highly urbanized area	778 (21.1)	779 (21.2)
Urbanized area	1407 (38.2)	1402 (38.2)
Moderately urbanized area	126 (3.4)	126 (3.4)
Lowly urbanized area	637 (17.3)	636 (17.3)
Rural area	735 (20.0)	732 (19.9)
Socio-economic status[e]		
High	1031 (28.0)	1031 (28.0)
Intermediate	1144 (31.1)	1142 (31.1)
Low	1508 (40.9)	1502 (40.9)
Education level[f]		
None	133 (3.6)	132 (3.6)
Low	869 (23.6)	869 (23.7)
Intermediate	1408 (38.2)	1408 (38.2)
High	945 (25.7)	943 (25.7)
Very high	263 (7.1)	263 (7.2)
Unknown	65 (1.8)	65 (1.8)
Net monthly income (€)		
<1150	549 (14.9)	549 (14.9)
1151–1750	748 (20.3)	745 (20.3)
1751–3050	1039 (28.2)	1036 (28.2)
>3051	574 (15.6)	573 (15.6)
Unknown	773 (21.0)	772 (21.0)
Variables subject to stepwise selection		
Gardened/handled soil/sand with bare hands (past 12 months)		
No	1061 (28.8)	1060 (28.8)
Yes	2622 (71.2)	2615 (71.2)
Eaten soil/sand (geophagia)		
No	3574 (97.1)	3567 (97.1)
Yes	109 (2.9)	108 (2.9)
Suffering from hay fever		
No	3203 (87.0)	3195 (86.9)
Yes	480 (13.0)	480 (13.1)
Suffering from celiac disease		
No	3675 (99.8)	3667 (99.8)
Yes	8 (0.2)	8 (0.2)
Suffering from eczema		
No	3301 (89.6)	3294 (89.6)
Yes	382 (10.4)	381 (10.4)
Suffering from asthma/COPD		
No	3453 (93.8)	3446 (93.8)
Yes	230 (6.2)	229 (6.2)

Table 6 (continued)

Variables always retained in the models	*Toxocara* N (%)	*Ascaris* N (%)
Suffering from lactose intolerance		
No	3616 (98.2)	3608 (98.2)
Yes	67 (1.8)	67 (1.8)
Suffering from egg allergy		
No	3677 (99.8)	3669 (99.8)
Yes	6 (0.16)	6 (0.2)
Suffering from peanut allergy		
No	3650 (99.1)	3642 (99.1)
Yes	33 (0.9)	33 (0.9)
Suffering from nut allergy		
No	3640 (98.8)	3632 (98.8)
Yes	43 (1.2)	43 (1.2)
Suffering from fish allergy		
No	3671 (99.7)	3663 (99.7)
Yes	12 (0.3)	12 (0.3)
Suffering from shellfish allergy		
No	3666 (99.5)	3658 (99.5)
Yes	17 (0.5)	17 (0.5)
Suffering from soya allergy		
No	3673 (99.7)	3665 (99.7)
Yes	10 (0.3)	10 (0.3)
Suffering from other grain allergy		
No	3661 (99.4)	3653 (99.4)
Yes	22 (0.6)	22 (0.6)
Suffering from other food allergy		
No	3592 (97.5)	3584 (97.5)
Yes	91 (2.5)	91 (2.5)
Travel to Asia, Africa, South or Central America for work		
No	3584 (97.3)	3576 (97.3)
Yes	99 (2.7)	99 (2.7)
Travel to Asia, Africa, South or Central America for family matters		
No	3401 (92.3)	3393 (92.3)
Yes	282 (7.7)	282 (7.7)
Travel to Asia, Africa, South or Central America for holidays		
No	2721 (73.9)	2714 (73.9)
Yes	962 (26.1)	961 (26.1)
Travel time to Asia, Africa, South or Central America		
None	2388 (64.8)	2380 (64.8)
≤6 weeks	929 (25.2)	928 (25.2)
7 weeks–3 months	93 (2.5)	93 (2.5)
4–12 months	101 (2.7)	102 (2.8)
≥13 months	172 (4.7)	172 (4.7)
Travel to Asia, Africa, South or Central America for other reasons		
No	3574 (97.0)	3565 (97.0)
Yes	109 (3.0)	110 (3.0)
Ever been abroad		
No	1390 (37.7)	1389 (37.8)
Yes	2293 (62.3)	2286 (62.2)
Ever travelled to Asia		
No	2894 (78.6)	2887 (78.6)
Yes	789 (21.4)	788 (21.4)
Ever travelled to Africa		
No	3072 (83.4)	3065 (83.4)
Yes	611 (16.6)	610 (16.6)
Ever travelled to South or Central America		
No	3203 (87.0)	3194 (86.9)
Yes	480 (13.0)	481 (13.1)
Owned cattle (past 5 years)		
No	3600 (97.8)	3592 (97.7)
Yes	83 (2.2)	83 (2.3)
Owned pigs (past 5 years)		
No	3659 (99.4)	3651 (99.4)
Yes	24 (0.6)	24 (0.6)
Owned sheep (past 5 years)		
No	3589 (97.5)	3581 (97.4)
Yes	94 (2.6)	94 (2.6)
Owned goat (past 5 years)		
No	3626 (98.5)	3618 (98.5)
Yes	57 (1.5)	57 (1.5)
Owned cats (past 5 years)		
No	2678 (75.2)	2764 (75.2)
Yes	915 (24.8)	911 (24.8)
Owned dogs (past 5 years)		
No	2831 (76.9)	2827 (76.9)
Yes	852 (23.1)	848 (23.1)
Owned poultry (past 5 years)		
No	3514 (95.4)	3507 (95.4)
Yes	169 (4.6)	168 (4.6)
Owned fish (past 5 years)		
No	3134 (85.1)	3128 (85.1)
Yes	549 (14.9)	547 (14.9)
Owned mice/hamsters (past 5 years)		
No	3600 (97.8)	3592 (97.7)
Yes	83 (2.2)	83 (2.3)
Owned rabbits (past 5 years)		
o	2962 (80.4)	2956 (80.4)
Yes	721 (19.6)	719 (19.6)
Owned other pets (past 5 years)		
No	3598 (97.7)	3590 (97.7)
Yes	85 (2.3)	85 (2.3)
Owned/being exposed to cats (past 5 years)		
No	1546 (42.0)	1543 (42.0)
Yes	2137 (58.0)	2132 (58.0)
Eaten raw unwashed vegetables (last 12 months)		
Never	2667 (72.4)	2662 (72.5)
Daily	110 (3.0)	111 (3.0)
Weekly	491 (13.3)	490 (13.3)
Monthly	219 (6.0)	217 (5.9)
Less than monthly	196 (5.3)	195 (5.3)
Eaten raw meat (last 12 months)		
Never	1702 (46.2)	1698 (46.2)
Daily	19 (0.5)	19 (0.5)
Weekly	450 (12.2)	449 (12.2)
Monthly	613 (16.6)	613 (16.7)
Less than monthly	772 (21.0)	769 (20.9)
Unknown	127 (3.5)	127 (3.5)
Family situation		
Married/registered partnership	1607 (43.7)	1606 (43.7)
Cohabiting	210 (5.7)	210 (5.7)
Single, never married	545 (14.8)	544 (14.8)
Single, divorced	126 (3.4)	125 (3.4)
Widow(er)	119 (3.2)	119 (3.2)
Unknown/not applicable	1076 (29.2)	1071 (29.2)
Children in the house attending day care centres		
No	3242 (88.0)	3235 (88.0)
Yes	305 (8.3)	305 (8.3)
Unknown/not applicable	136 (3.7)	135 (3.7)
Playing in a sandpit		
No	473 (12.8)	473 (12.9)
Yes	528 (14.3)	523 (14.2)
Unknown/not applicable	2682 (72.8)	2679 (72.9)
Average weekly time spent playing in a sandpit		
≤1 h	176 (4.8)	175 (4.8)
1–3 h	142 (3.9)	141 (3.8)
>3 h	169 (4.6)	167 (4.5)
Unknown/not applicable	3196 (86.8)	3192 (86.9)
Sandpit at home		
No	3458 (93.9)	3453 (94.0)

Table 6 (continued)

Variables always retained in the models	*Toxocara* N (%)	*Ascaris* N (%)
Yes	225 (6.1)	222 (6.0)
Sandpit at school		
No	3298 (89.6)	3294 (89.6)
Yes	385 (10.5)	381 (10.4)
Sandpit at public park		
No	3431 (93.2)	3426 (93.2)
Yes	252 (6.8)	249 (6.8)

[a] Defined as being born in the Netherlands and having both parents being born in the Netherlands as well

[b] Any Western country other than the Netherlands in Europe, North America, Australia and New Zealand

[c] Any non-Western country other than Morocco, Turkey, Surinam and Netherlands Antilles

[d] Expressed as addresses/km^2 per postcode: highly urbanized area >2000 addresses/km^2, urbanized area 1500–2000 addresses/km^2, moderately urbanized area 1000–1500 addresses/km^2, lowly urbanized area 500–1000 addresses/km^2 and rural area <500 addresses/km^2

[e] Expressed as a normalized score ranging from −4 to +4 based on income, employment and educational level per postcode area. Categorized according to tertiles of the socio-economic status score distribution in the whole of the Netherlands

[f] None = no education; low = primary, lower vocational or lower secondary education; intermediate = intermediate vocational or intermediate secondary education; high = higher secondary or higher vocational education; very high = university education

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