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1 ***Toxocara* and *Ascaris* seropositivity among patients suspected of visceral and**  
2 **ocular larva migrans in the Netherlands: trends from 1998 to 2009**

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10

11 **Running title:** Trends of *Toxocara* and *Ascaris* seropositivity in the Netherlands

12

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15

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1 **Abstract**

2 *Toxocara canis*, *Toxocara cati* and *Ascaris suum* are roundworms of dogs, cats and  
3 pigs respectively that can also infect humans. These zoonotic helminths have a  
4 worldwide distribution and are also endemic in the Netherlands. Infection with  
5 *Toxocara sp.* may result in visceral larva migrans (VLM) or ocular larva migrans  
6 (OLM) caused by the migrating larvae. Although *A. suum* has been reported to  
7 occasionally mature to the adult stage in humans, clinical cases of VLM suspected to  
8 be caused by *A. suum*, has been described. Diagnosis of these helminth infections  
9 relies mainly on serology. Here we analyse the results from the *Toxocara* and *Ascaris*  
10 IgG-ELISA from a total of 2838 serum samples from VLM and OLM suspected  
11 patients that were sent to our institution from 1998 to 2009. Results indicate that for  
12 each year the *Ascaris* seropositivity is significantly higher compared to the *Toxocara*  
13 seropositivity. Furthermore, while *Toxocara* seropositivity has decreased in time, the  
14 *Ascaris* seropositivity has not significantly changed for the past 12 years. The *Ascaris*  
15 and *Toxocara* seropositivity was also shown to increases with age and while gender  
16 has no influence on the *Ascaris* seropositivity, males showed higher *Toxocara*  
17 seropositivity.

18

19

1 **Introduction**

2 Dogs, cats and pigs are the final hosts for *Toxocara canis*, *Toxocara cati* and *Ascaris*  
3 *suum* respectively. Female worms present in the intestine of these animals shed large  
4 numbers of eggs that together with the feces contaminate the environment. The eggs  
5 of *Toxocara sp.* and *Ascaris suum* become infectious only after embryonation in soil,  
6 which at optimal temperature and humidity occurs within 4 to 6 weeks [1,2].

7 Transmission of both *Toxocara sp.* and *Ascaris suum* to humans usually takes place  
8 after accidental ingestion of larvae-containing eggs. Young children with geophagia  
9 (deliberate ingestion of soil) are at highest risk, but can also occur in adults [3].

10 People can also become infected with *Toxocara canis* by eating unwashed vegetables  
11 grown in contaminated soil and by consumption of raw or undercooked meat from  
12 potential paratenic hosts such as chicken and lambs [4,5]. Transmission of *Ascaris*  
13 *suum* by ingestion of liver and lungs from infected chickens has also been described  
14 [6]. After ingestion of contaminated soil or food, *Toxocara* larvae reach the  
15 gastrointestinal tract, penetrate the intestines and migrate throughout the body to  
16 various organs such as the liver and lungs. Humans are accidental host for *Toxocara*  
17 *sp.* and the larvae do not reach the adult stage. Few studies have reported that in some  
18 cases *A. suum* can develop to adult worms in the human host [7,8]. However, others  
19 have reported on clinical VLM cases suspected to be caused by *A. suum* with larval  
20 migration and pathology with no mention of the larvae maturing to the adult stage  
21 [9,10].

22 Although usually asymptomatic, infection with *T. canis* can cause in humans a  
23 syndrome known as visceral larva migrans (VLM) which is characterized by fever,  
24 pulmonary infiltrates, coughs, neurological distress, hyperglobulinemia and  
25 hepatomegaly. Eosinophilic pneumonitis (Loeffler's pneumonia) which has a clinical

1 resemblance to pulmonary inflammation observed in asthmatic patients, has been  
2 described [11]. One serious complication is migration of the larvae to the eyes causing  
3 ocular larva migrans (OLM) often resulting in granulomatous reaction in the retina,  
4 leading in the worst cases to blindness. The brain, heart, and other organs can also be  
5 affected [12].

6 Diagnosis of VLM and OLM relies mainly on serology. The most recommended  
7 serological assay used is the ELISA and the ES (excretory/secretory) antigen derived  
8 from the migrating larvae. For nearly thirty years we have been using the same  
9 ELISA procedure [13] based on the ES antigen derived from *Toxocara canis* or  
10 *Ascaris suum* larvae.

11 To study the importance of exposure to these zoonotic helminths for the public health  
12 we determined the trends of *Toxocara* and *Ascaris* seropositivity among patients  
13 suspected of suffering from VLM or OML over the past 12 years in the Netherlands.  
14 Age and gender distribution of the observed seropositivity was also investigated.

15  
16

1 **Materials and Methods**

2

3 **Serum samples**

4 A total of 2838 serum samples from suspected VLM and OLM patients sent to our  
5 institution from 1998 to 2009 were used in this study. We are the only institution in  
6 the Netherlands that routinely carries out serology for these worm infections and  
7 serum samples from different hospitals throughout the country are referred to us.

8 Since *Ascaris* and *Toxocara* infections can not be clinically distinguished from each  
9 other, serum samples from VLM and OLM suspected patients are routinely tested in  
10 both the *Toxocara* and *Ascaris* IgG-ELISA. Information on clinical manifestations  
11 and laboratory examination of these patients are usually not reported to our institution  
12 however when reported, they include eosinophilia, malaise, fever, tiredness,  
13 respiratory complaints, diarrhea, pneumonia, abdominal pain and/or uveitis.

14

15 **Preparation of *Toxocara canis* and *Ascaris suum* excretory/secretory antigen**

16

17 The excretory-secretory (E/S) antigen derived from *Toxocara canis* and *Ascaris suum*  
18 was prepared as previously described [14]. Briefly, adult *T. canis* worms were  
19 collected from the faeces of naturally infected dogs and *A. suum* from the intestine of  
20 pigs kindly provided by a slaughter house. Eggs were collected from the uteri of  
21 female worms and were allowed to embryonate in 0.05 M H<sub>2</sub>SO<sub>4</sub> in the dark at room  
22 temperature for 4-6 weeks. Embryonated eggs were stored in 0.05 M H<sub>2</sub>SO<sub>4</sub> at 4 °C  
23 until use.

24 Larvae were freed from the egg shells and allowed to migrate through cotton wool  
25 contained in Pasteur pipets that were placed in tubes filled with medium, at 37°C  
26 overnight. The migrating larvae were collected and counted. A suspension of 150

1 larvae per ml medium was incubated at 37°C. After 24 h the medium was discarded  
2 due to the content of high levels of phosphorylcholine which were determined using  
3 an ELISA [14]. Fresh medium was added and after a week the harvested medium was  
4 used as the E/S antigen. The content of crossreactive components such as  
5 phosphorylcholine and tropomyosin were shown for *T. canis* and *A. suum* ES [14], to  
6 be below the detection limit of the respective assays.

7

### 8 **Detection of anti-*Toxocara* and *Ascaris* IgG antibodies**

9 Detection of anti-*Toxocara* and *Ascaris* IgG antibodies was performed using an  
10 ELISA and the excretory/secretory (E/S) antigen derived either from *T. canis* or *A.*  
11 *suum* larvae. Medium binding ELISA microtiter plates (Nunc, Roskilde, Denmark)  
12 were used for the *Toxocara* ELISA and high binding plates (Greiner, Frickenhausen,  
13 Germany) were used for the *Ascaris* ELISA as previously described [14]. The plates  
14 were coated with E/S antigen (10 µg/ml) diluted in 0.1 M sodium carbonate  
15 (Na<sub>2</sub>CO<sub>3</sub>), pH 9.6. Plates were incubated overnight (without lids) at 37 °C to allow the  
16 E/S antigen to dry on to the wells. Plates were washed three times with phosphate-  
17 buffered saline (pH 7.2) containing 0.05% v/v Tween-20 (PBS/Tween). For the  
18 *Ascaris* ELISA an additional blocking step was performed by adding to every well 2  
19 % bovine serum albumin (BSA) (Boehringer Mannheim, GmbH, Germany) solution  
20 in PBS/Tween. Plates were incubated for 30 min. at 37 °C and thereafter washed three  
21 times with PBS/Tween. Serum samples were diluted 1:40 in 2% BSA/PBS/Tween and  
22 added to the plates. After 1h incubation at 37 °C, plates were washed and anti-human  
23 IgG conjugated to alkaline phosphatase (DAKO, Glostrup, Denmark) diluted in 4 %  
24 BSA/PBS/Tween were added for 1 h at 37 °C. After plates were washed, substrate,  
25 H<sub>2</sub>O<sub>2</sub>, 0.05% and 5-ASA was added for 1 h at room temperature after which the

1 absorbance was read at 450 nm. The extinction value of the tested serum and of the  
2 cut-off serum was used to calculate a ratio. A ratio higher or equal to 1.0 was  
3 considered positive. The cut-off value was defined as the mean absorbance of 20  
4 serum samples from healthy blood donors plus three times the standard deviation.

5

### 6 **Specificity and Sensitivity of the *Toxocara* and *Ascaris* ELISA**

7 The specificity of the *Toxocara* and *Ascaris* ELISA was determined using sera from  
8 patients with other parasitic and bacterial infections: *Toxoplasma gondii* (n=10),  
9 *Taenia solium* (n=14), *Trichinella sp.*(n=10), *Echinococcus granulosus.*(n=10),  
10 *Leishmania sp.* (n=10) *Entamoeba histolytica* (n=10), *Borrelia sp.*(n=10), *Treponema*  
11 *pallidum* (n=10) and *Bordetella pertusis* (n=100). Results indicate a specificity of 89  
12 % for the *Toxocara* IgG-ELISA and 76% for the *Ascaris* IgG-ELISA [14]. Since sera  
13 from patients parasitologically confirmed for *Ascaris suum* infections were not  
14 available, sensitivity of this ELISA could not be determined. The sensitivity of the  
15 *Toxocara* ELISA was calculated using serum samples from patients with toxocariasis  
16 diagnosed based on clinical disease, hematological and serological findings . The  
17 sensitivity of the *Toxocara* ELISA was calculated to be 82%. The quality of the  
18 *Toxocara* IgG ELISA is since 2006 periodically evaluated by an external quality  
19 control assessment organized by the UK NEQAS Microbiology, with so far a 100%  
20 correct score.

21

### 22 **Statistical methods**

23 In order to identify seropositivity trends over the past twelve years and differences in  
24 age and gender of subjects testing positive for *Ascaris sp.* or *Toxocara sp.* univariate  
25 models were developed using logistic regression. Differences were considered



- 1 significant at p values of  $\leq 0.05$ . In the results odds ratios and their 95% confidence
- 2 intervals are given.
- 3

1 **Results**

2

3 ***Toxocara* and *Ascaris* seropositivity from 1998 to 2009**

4 From 1998 to 2009 a total of 2838 serum samples from patients suspected of a  
5 *Toxocara* or *Ascaris* infection were received at the RIVM. On average 237 serum  
6 samples ranging from a minimum of 206 to a maximum of 315 samples were sent  
7 yearly to our laboratory with request for *Toxocara* and *Ascaris* serology. Figure 1  
8 shows the distribution in time of the seropositivity for these helminths. On average  
9 33% (95% C.I. 31-35%) were found to be *Ascaris* positive while 8% (95% C.I. 7-9%)  
10 was *Toxocara* positive. The percentage of *Ascaris* positive patients varies from 24.5%  
11 to 47.0% and fluctuates over the years. From 1998 to 2009 the *Ascaris* seropositivity  
12 did not change significantly (Table 1a). In contrast, the percentage of *Toxocara*  
13 positive patients has significantly decreased over time ( $p < 0.01$ ) from a maximum of  
14 17.7% in 1998 to a minimum of 4.0% in 2002 (Table1b). From 2006 to 2009 a  
15 significant ( $p < 0.05$ ) increase in the seropositivity was observed. However, in 2009  
16 the *Toxocara* seropositivity was 11.2% which was still lower compared to 1998  
17 (Table1b).

18

19 **Age distribution of *Toxocara* and *Ascaris* seropositivity**

20 Figure 2 shows the age distribution of the number of serum samples send to our  
21 laboratory from 1998 to 2009 with the request for *Toxocara* and *Ascaris* serology. For  
22 the age groups of 60 years and older there was a smaller number of serum samples  
23 compared to the other age groups.

24 The age distribution of *Toxocara* and *Ascaris* seropositivity is shown in Fig. 3. The  
25 percentage of *Ascaris* seropositivity shows a significant ( $p < 0.01$ ) increase with age,

1 ranging from 19% for the age group 0-10 years up to 50% for the age group of 70  
2 years and older. This results in an odds ratio of 4.3 (CI 2.8-6.7) for the age group of  
3 70 years and older as compared to the 0-10 years age group. The *Toxocara*  
4 seropositivity shows a different distribution, with the age group 0-10 showing a  
5 slightly higher seropositivity (8%) compared with the age group of 21-30  
6 (seropositivity: 6%). This difference is however not significant. The seropositivity  
7 thereafter clearly increases with age reaching 15% for the age group of 61-70 years  
8 ( $p < 0.01$ , OR 1.9 CI 1.3-3.1). Since the age group 0-10 is rather broad, the same  
9 analysis was performed for intervals of 5 years. Comparable results were found,  
10 namely that for age group 0-5 (8.4%, 95% C.I. 5.3-13.2%) and 6-10 (7.9%, 95% C.I.  
11 4.9-12.5%) a higher seropositivity was found compared to the age group 11-15 (4.8%,  
12 95% C.I. 2.5-8.8%), but the differences were not significant.

13

#### 14 **Gender difference in the *Toxocara* and *Ascaris* seropositivity**

15 The possible gender effect on seropositivity for these helminths was also analysed.  
16 Fig 4a shows that gender has no influence on *Ascaris* seropositivity. In contrast males  
17 show significantly higher *Toxocara* seropositivity compared to females ( $p < 0.01$ , OR  
18 1.6, CI 1.2-2.1). The effect of gender and age on *Toxocara* seropositivity was also  
19 analysed. Since the *Toxocara* seropositivity for the age group 0 to 20 years was  
20 slightly higher compared to 21 to 40 years (Fig3b) we analysed the effect of gender  
21 for patients below and above 20 years of age. Fig 4b shows that the increase of  
22 seropositivity with age is especially marked in males, with the age group of 21 and  
23 older having a significantly higher *Toxocara*-seropositivity ( $p < 0.05$ , OR 1.7, CI 1.1-  
24 2.7).

## 1 **Discussion**

2 Zoonotic helminths such as *A. suum*, *T. canis* and *T. cati* have a cosmopolitan  
3 distribution and toxocariasis has been described as one of the most common zoonotic  
4 infections throughout the world [15].

5 Different techniques and parasite antigens have been used for serodiagnosis of  
6 *Toxocara* and *Ascaris* infections. The ELISA based on the use of the  
7 excretory/secretory (ES) antigens produced by the larvae of these helminths is the  
8 most common approach for serodiagnosis [15,18]. In this study we describe an ELISA  
9 based on the ES products from *T. canis* and *A. suum* larvae that has been used in our  
10 laboratory for nearly thirty years [13]. It is important to realize that due to  
11 crossreactivity the *Toxocara* ELISA can not distinguish between a *T. canis* or *T. cati*  
12 infections and that the *Ascaris* ELISA can not distinguish between infections with *A.*  
13 *suum* and *A. lumbricoides*. However, *A. lumbricoides* infections are practically absent  
14 in the Netherlands as indicated from two different population studies on  
15 gastroenteritis where stool samples from dutch patients and healthy controls were  
16 examined for different intestinal parasites and no helminth eggs were found [19,20].  
17 Although *Toxocara sp.* and *Ascaris sp.* have antigens in common that can lead to  
18 crossreactivity [21] we have previously shown that in the *A. suum* ES products the  
19 content of crossreactive components such as phosphorylcholine and tropomyosin are  
20 below the detection limit of the respective assays [14]. The content of these  
21 components were also shown to be below the detection limits in the *T. canis* ES  
22 products (data not shown).

23 Both of these zoonotic infections are endemic in the Netherlands and although  
24 crossreactivity is commonly suggested, results from this study show clear differences  
25 in the percentage of *Toxocara* or *Ascaris* seropositivity in the past 12 years.

1 Meanwhile, the overall *Toxocara* seropositivity among the patients suspected of VLM  
2 or OLM is 8 % the *Ascaris* seropositivity is 4 times higher. In addition, the trends of  
3 seropositivity over the years is also different for both pathogens. For the past twelve  
4 years a decrease in the *Toxocara* seropositivity is observed compared to no changes  
5 for the *Ascaris* seropositivity.

6 Campaigns on deworming dogs and cats exists for many years in the Netherlands  
7 which is one of the recommended measures for control of animal and human  
8 toxocariasis [22,23]. Recently, up-to-date guidelines for deworming and prevention  
9 of parasitic infections in pets and zoonotic infections in man has become available  
10 (<http://www.esccap.org/>). This might explain the observed decrease of *Toxocara*  
11 seropositivity in the past twelve years. Very little however, is known about ascariasis  
12 in humans due to *A. suum*. The Netherlands is one of the largest pig producers in  
13 Europe and from a study carried out in this country on the prevalence of  
14 gastrointestinal parasites in pigs it has been shown that infections with *A. suum* is  
15 very common, particularly in pigs with outdoor facilities [24]. What is still unknown,  
16 is the route of transmission of this helminth to the human population. Early studies  
17 have shown the presence of *A. suum* eggs in sewage sludge which has been widely  
18 used as fertilizer on agricultural grounds, public parks and potting compost usually  
19 used for gardening [18]. These authors suggest that this contaminated soil could be de  
20 source of *Ascaris* infections in the Netherlands. Exposure to *A. suum* could therefore  
21 affect not only persons working in pig farms but also those living in other areas.  
22 Whether exposure to these helminths in the Netherlands is also due to consumption of  
23 meat from infected pigs and intermediate hosts such as chickens and cattle remains to  
24 be investigated.

1 What is also interesting from the data presented here is that although we receive  
2 comparable number of serum samples from patients from different age groups, the  
3 percentage of *Toxocara* seropositive children in the age group 0 to 10 is slightly  
4 higher compared with age groups 11 to 40. This could be explained by the fact that  
5 young toddlers usually put things in their mouth including sand from sandpits or soil  
6 from playgrounds or backyards that can be contaminated with *Toxocara* eggs.

7 The *Ascaris* seropositivity increased with age to more than 50% for the age group of  
8 70 and older, indicating a continuous exposure to this helminth. Since all serum  
9 samples here analyzed were tested in both *Toxocara* and *Ascaris* ELISAs, the  
10 difference in seropositivity for these helminths in the age group of 1 to 10 years  
11 compared with the other age groups might indicate differences in the route of  
12 transmission. Whether *Ascaris* transmission for this age group is food-borne rather  
13 than by direct ingestion of contaminated soil remains to be investigated.

14 The influence of gender was also found to be different between the *Ascaris* and  
15 *Toxocara* seropositivity. While gender had no effect on the *Ascaris* seropositivity,  
16 males showed a higher *Toxocara* seropositivity. This has been previously reported for  
17 male children from different seroprevalence studies [25,26]. However, here we show  
18 that males older than 20 years were the ones that had a higher *Toxocara*  
19 seropositivity. Whether this finding has an association with the occupational history  
20 of the male patients is a question that could be addressed in future studies. Deutz *et al.*  
21 carried out a study on the risk of *Toxocara* infections for several occupational groups  
22 and they found that farmers showed the highest *Toxocara* seroprevalence (44%),  
23 followed by veterinarians (27%), slaughterhouse staff (25%) and hunters (17%),  
24 whereas only 2% of the individuals of the control group were seropositive. The

1 authors suggest that the main source of infection in rural areas appears to be roaming  
2 of farm cats and dogs that have not been dewormed [27].

3 Several studies on the epidemiology, pathology and diagnosis of toxocariasis are  
4 available [15,28] however, much less is known about human infections with *A. suum*.  
5 Few studies have reported on *Ascaris suum* being able to mature to the adult stage in  
6 the human host [7,8,29]. Neijsum et al refer to this infection as zoonotic ascariasis and  
7 have recently reported that *A. suum* can also mature to the adult stages in chimpanzees  
8 [30]. There are however other studies reporting VLM cases that are suspected to be  
9 caused by *Ascaris suum* in which pulmonary and liver lesions have been described  
10 [9,10]. As suggested by Arizono et al. the pathogenic or physiogenetic factors that  
11 determine the course of human infection with pig-derived *Ascaris* remain to be  
12 elucidated [8].

13 Recently, we have shown a positive association between *Ascaris* seropositivity and  
14 allergic manifestations in 4-year old children in the Netherlands [14]. In an earlier  
15 study carried out with elementary Dutch school children it was suggested that allergic  
16 manifestations occur more often in *Toxocara*-seropositive children. Studies in other  
17 countries have also reported that *Toxocara* infections may be associated with an  
18 increased risk for allergic manifestations [31]. Using murine models we have shown  
19 that indeed infection with *T. canis* results in exacerbation of experimental airway  
20 inflammation [32].

21 Clinical awareness and diagnosis of infection with these helminths is essential, not  
22 only because of the syndromes that they may cause but also to prevent exacerbation of  
23 ongoing allergic manifestations which has increased in the past decades throughout  
24 the world. Furthermore, identification of the source of infection and proposing

1 preventive measures to avoid contamination of the environment with these helminth  
2 eggs are of great important for public health.

3 In this study we describe a *Toxocara*- and *Ascaris*-ELISA and an overview of the  
4 seropositivity for these roundworms in the past twelve years in the Netherlands.

5 Results indicate a decrease in the *Toxocara* but not in the *Ascaris* seropositivity.

6 Epidemiological studies must be carried out in order to determine risk factors for  
7 infection and propose appropriate preventing measures against infection with these  
8 roundworms.

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Table 1  
Number and percentage of *Ascaris* (a) and *Toxocara* (b) positive samples per year, with corresponding odds ratios, confidence intervals and p-values calculated by logistic regression using 1998 as a reference.

Table 1a

<b>Ascaris</b>							
Year	no. samples	no. positive	% positive	OR	lowerCI	upperCI	p-value
1998	235	92	39.1%	reference			
1999	259	70	27.0%	<b>0.58</b>	<b>0.39</b>	<b>0.84</b>	<b>&lt;0.01</b>
2000	315	101	32.1%	0.73	0.52	1.04	0.09
2001	217	102	47.0%	1.38	0.95	2.01	0.09
2002	253	62	24.5%	<b>0.50</b>	<b>0.34</b>	<b>0.74</b>	<b>&lt;0.001</b>
2003	242	85	35.1%	0.84	0.58	1.22	0.36
2004	234	74	31.6%	0.72	0.49	1.05	0.09
2005	209	62	29.7%	<b>0.66</b>	<b>0.44</b>	<b>0.97</b>	<b>0.04</b>
2006	206	63	30.6%	0.68	0.46	1.02	0.06
2007	217	85	39.2%	1.00	0.69	1.46	1.00
2008	225	66	29.3%	<b>0.65</b>	<b>0.44</b>	<b>0.95</b>	<b>0.03</b>
2009	219	75	34.2%	0.81	0.55	1.19	0.28

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Table 1b

<b>Toxocara</b>							
Year	no. samples	no. positive	% positive	OR	lowerCI	upperCI	p-value
1998	238	42	17.7%	reference			
1999	259	40	15.4%	0.85	0.53	1.37	0.51
2000	315	26	8.3%	<b>0.42</b>	<b>0.25</b>	<b>0.70</b>	<b>&lt;0.01</b>
2001	216	14	6.5%	<b>0.32</b>	<b>0.17</b>	<b>0.60</b>	<b>&lt;0.001</b>
2002	253	10	4.0%	<b>0.19</b>	<b>0.09</b>	<b>0.38</b>	<b>&lt;0.001</b>
2003	243	10	4.1%	<b>0.20</b>	<b>0.09</b>	<b>0.39</b>	<b>&lt;0.001</b>
2004	234	16	6.8%	<b>0.34</b>	<b>0.18</b>	<b>0.62</b>	<b>&lt;0.001</b>
2005	210	13	6.2%	<b>0.31</b>	<b>0.15</b>	<b>0.58</b>	<b>&lt;0.001</b>
2006	206	9	4.4%	<b>0.21</b>	<b>0.10</b>	<b>0.43</b>	<b>&lt;0.001</b>
2007	217	12	5.5%	<b>0.27</b>	<b>0.13</b>	<b>0.52</b>	<b>&lt;0.001</b>
2008	227	18	7.9%	<b>0.40</b>	<b>0.22</b>	<b>0.71</b>	<b>&lt;0.01</b>
2009	214	24	11.2%	<b>0.59</b>	<b>0.34</b>	<b>1.00</b>	<b>0.05</b>

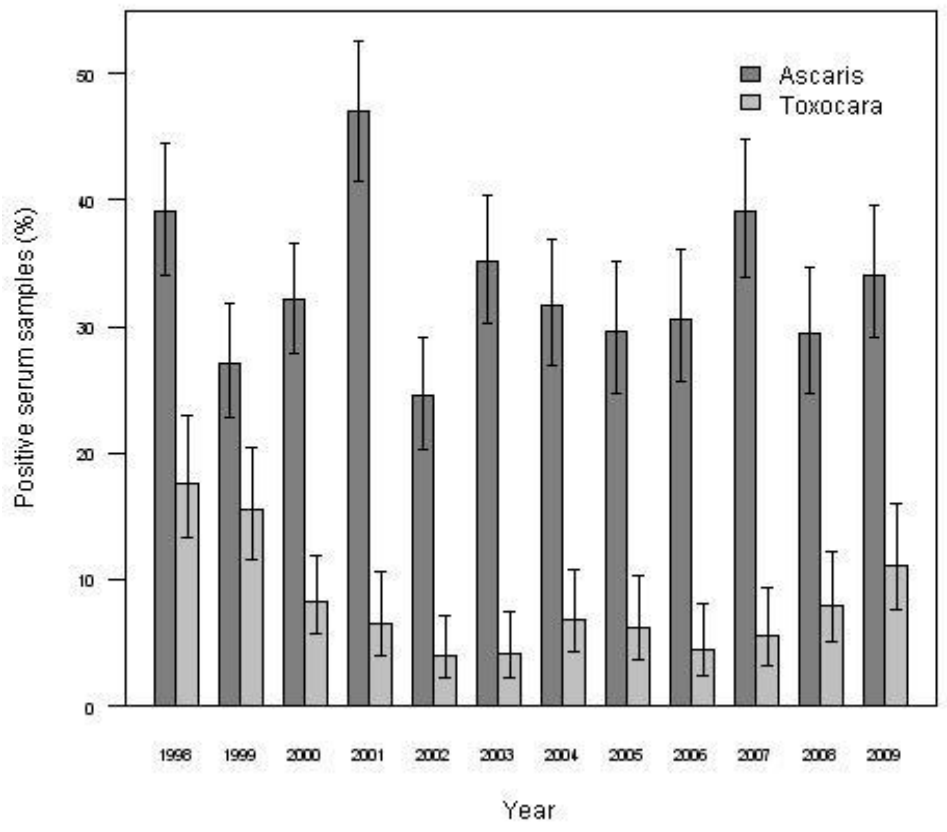
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2 Fig 1. Percentage of *Ascaris* or *Toxocara* IgG positive patients from 1998 to 2009

3 Bars represent seropositivity with their 95% confidence intervals given by the error

4 bars.



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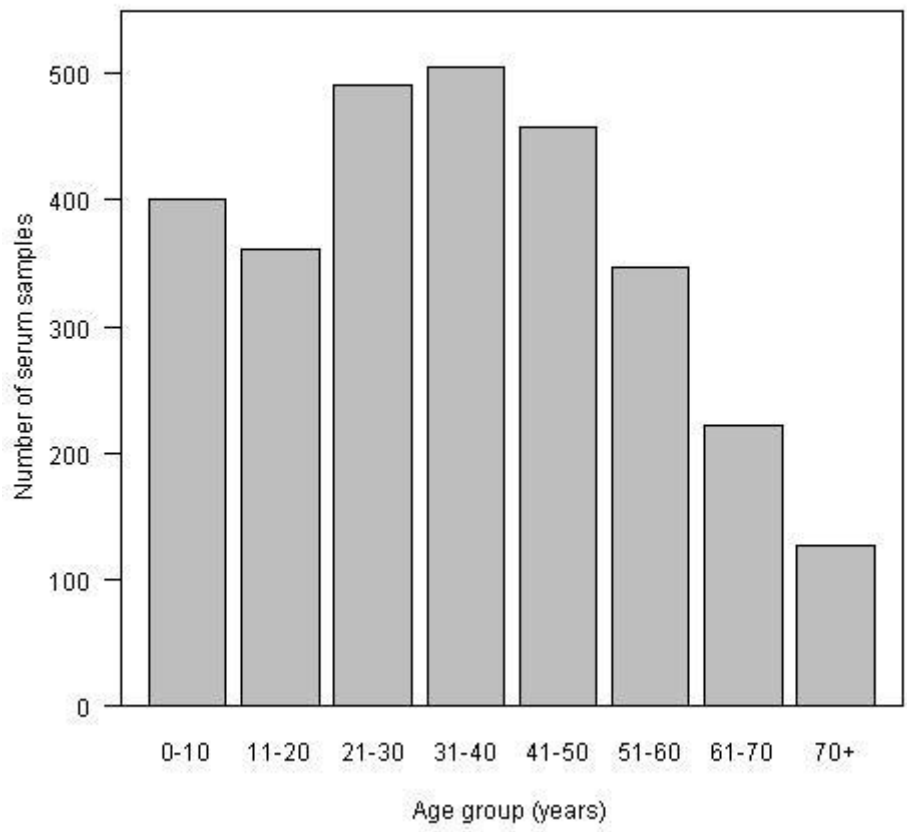
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2 Fig. 2. Age distribution for number of serum samples received at the RIVM from  
3 1998 to 2009 with request for *Toxocara* and *Ascaris* serology.

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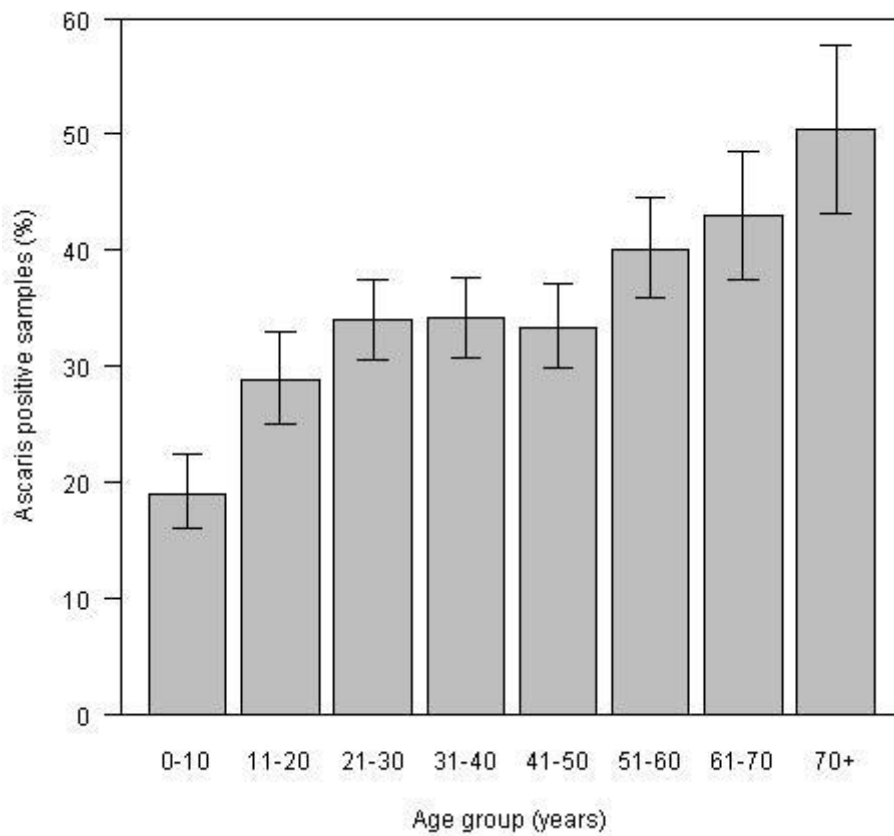
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1 Fig 3. Age distribution of percentage *Ascaris* (a) or *Toxocara* (b) IgG positive sera  
2 received at the RIVM from 1998 to 2009. Bars represent seropositivity with their 95%  
3 confidence intervals given by the error bars.

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5 Fig. 3a



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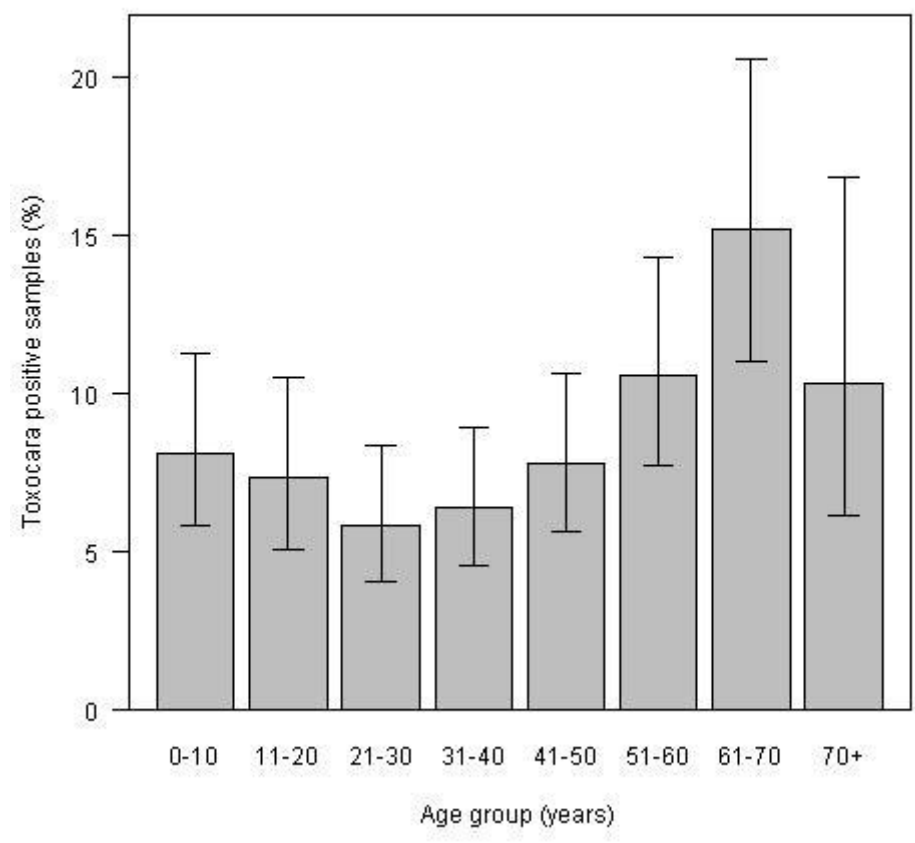
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2 Fig. 3b

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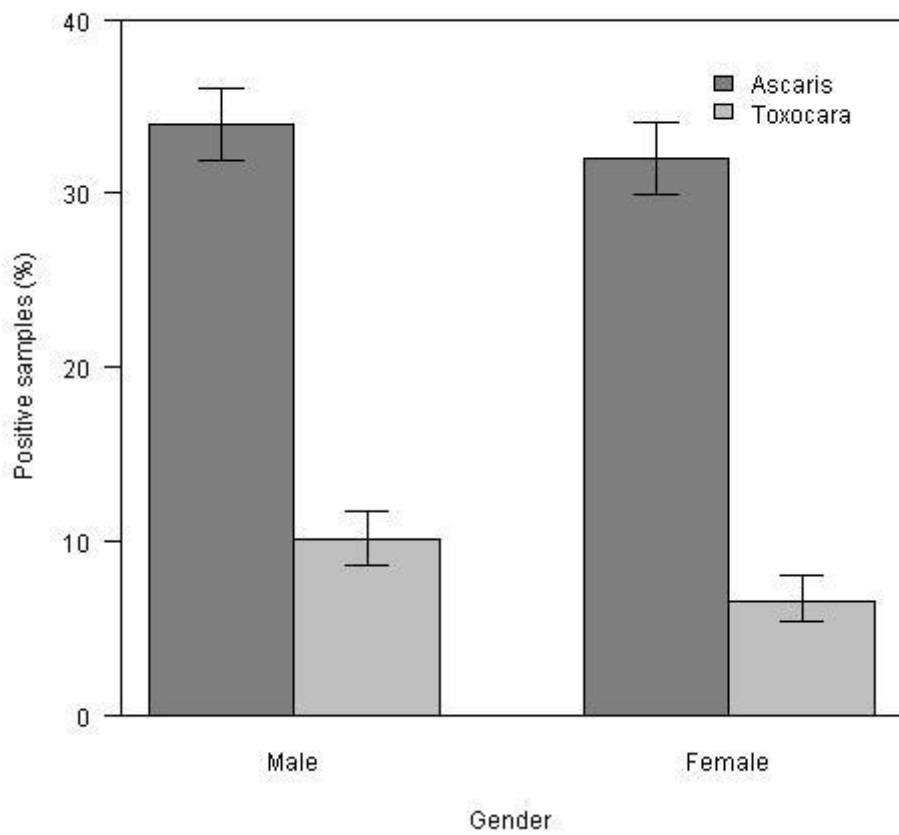
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1 Fig 4. Gender distribution of the (a) percentage *Ascaris* or *Toxocara* IgG positive sera  
2 received at the RIVM from 1998 to 2009 and (b) its association with age. Bars  
3 represent seropositivity with their 95% confidence intervals given by the error bars.

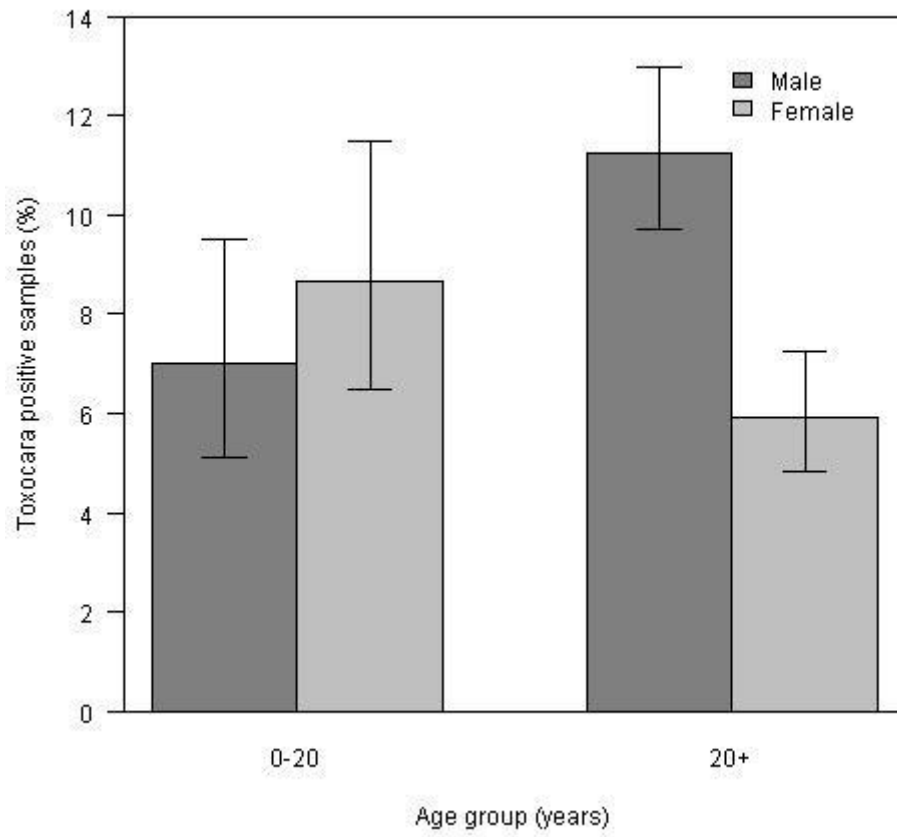
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6 Fig. 4a



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