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Population-based Toxoplasma seroprevalence study in The Netherlands

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SUMMARY

During 1995–1996 a population-based seroprevalence study was conducted in The Netherlands. Risk factors were established for postnatally acquired toxoplasmosis. The results were compared with a study conducted during 1987–1988 in pregnant women in the Southwest of The Netherlands in order to estimate the change in seroprevalence. In total, 7521 sera were tested and the national seroprevalence was 40·5% (95% CI 37·5–43·4). Living in the Northwest, having professional contact with animals, living in a moderately urbanized area, being divorced or widowed, being born outside The Netherlands, frequent gardening and owning a cat were independently associated with Toxoplasma seropositivity. Risk factors like eating undercooked meat could not be studied. The seroprevalence among women aged 15–49 years was 10% lower (35·2%, 95% CI 32·9–38·6) in the study of 1995–1996, compared to the Toxoplasma study of 1987–1988 (45·8%, 95% CI 45·2–46·3). The steepest rise in seroprevalence still occurred among the subjects aged 25–44 years.

INTRODUCTION

In Europe toxoplasmosis is one of the most common parasitic diseases in man. Human infection with *Toxoplasma gondii* can be acquired congenitally or postnatally. The transmission of Toxoplasma to humans occurs by ingestion of tissue cysts of intermediate hosts, by ingestion of inadequately cooked or raw meat, or by ingestion of oocysts. Oocysts are excreted by cats and can survive in the environment for a long period. Infection can occur by ingestion of contaminated soil after working in the garden or eating vegetables. Transmission of tissue cysts by organ transplantation can lead to severe complications enhanced by simultaneous immunosuppression therapy. Furthermore, vertical transmission in pregnancy can result in congenital infection [1]. Transmission of Toxoplasma by blood tranfusion has been reported [2, 3].

Most postnatally acquired infections are asymptomatic or present only with flu-like symptoms. In a minority of cases the infection is symptomatic. Although lymphadenopathy is the most common symptom, new knowledge about postnatally acquired ocular toxoplasmosis has led to insight that serious, recurrent eye disease is also one of the symptoms [4]. Furthermore, an infection during pregnancy can lead to severely affected children with retinochoroidal lesions, hydrocephalus and mental handicaps.

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However, most children will be asymptomatic at birth. Ten to twenty per cent of all congenitally infected children have manifested disease. Long-term follow-up showed that 80% of untreated infected children will develop ocular disease [5, 6].

Prevention of toxoplasmosis is hampered by uncertainty about how infection is acquired. Recently the results of risk-factor studies in pregnant women have been published [7, 8]. In the study by Buffolano and colleagues in Naples, eating cured pork and raw meat were the most important risk factors in pregnant women [9]. In a European multicentre case-control study carried out by the European Research Network on Congenital Toxoplasmosis, the most important risk factor was eating undercooked or raw meat, especially beef or lamb. Contact with soil and travel outside Europe and the United States and Canada were also risk factors [7]. In a prospective case-control study by Kapperud and colleagues in Norway, the most important risk factor for pregnant women was not just eating undercooked or raw meat, but also eating raw vegetables and fruit [8].

Seroprevalence rates vary in the different countries between 13% in the United Kingdom and Norway and 71% in France [10]. Most seroprevalence studies performed are conducted among women of reproductive age. In a study during 1987–1988 in The Netherlands the seroprevalence among pregnant women was 46%. This study, the Toxoplasma Intervention Prevention (TIP) study, was conducted in the southwest of The Netherlands, essentially the city of Rotterdam and its surroundings [11].

Approximately 8 years after this study we performed a population-based Toxoplasma seroprevalence study in the general Dutch population. The sera were obtained from a serum bank of the general Dutch population, aged <1–79 years [12], previously established for public health research. Sera were primarily used to evaluate effects of the national immunization programme. However, this serum bank also offered the opportunity to obtain insight into other infectious diseases, particularly those which frequently display a subclinical course. The data obtained by questionnaire, however, did not include information on eating habits.

Our main objectives were to study Toxoplasma seroprevalence in The Netherlands, compare the results with data from previous studies to establish whether seroprevalence was increasing or decreasing, and to determine risk factors for postnatally acquired toxoplasmosis.

MATERIALS AND METHODS

Study population

The study design and details on the data collection of this, the Pienter project, have been published elsewhere [12]. In short, to establish a serum bank of the general population in The Netherlands, eight municipalities with probabilities proportional to their population sizes were sampled within each of five geographical Dutch regions with similar population sizes. An age-stratified sample (age groups <1, 1–4, 5–9, … , 75–79 years) of 380 individuals was randomly selected from each municipality. Subjects were requested to give a blood sample and to complete a questionnaire. Samples and data were collected in the period from October 1995 to December 1996. The participation rate was 55% in the nationwide sample.

In the TIP study, pregnant women from Rotterdam and its surroundings participated. Pregnant women were invited by their general practitioner, obstetrician or midwife to participate in the study at the first antenatal pregnancy check, when they were no more than 20 weeks pregnant. All women received verbal information about Toxoplasma and a leaflet 'How to prevent a Toxoplasma infection' [11].

Antibody assay

The sera had been stored at −86 °C. Antibodies against *Toxoplasma gondii* were determined in a sandwich ELISA in a serum dilution of 1:20 (adapted to the method described by Ruitenberg and van Knapen [13]). The antigen is a crude *Toxoplasma* RH strain, the conjugate is a peroxidase-labelled anti-human IgG conjugate (Dako, Glostrup, Denmark). A cut-off serum was used and its optical density value was allowed to vary between 0·10–0·30. The methods, antigens and controls have not been changed over the last 15 years. Therefore, the results of the TIP study and our study are comparable. The extinction value of the tested serum and the cut-off serum was used to calculate a ratio. A ratio of <1 was considered to be negative; a ratio of at least 1·0 to be positive. Sufficient serum was available for 7521 of the 8359 participants. The missing participants consisted mostly of children <1 year old because a smaller amount of serum was collected for these children. Serum was used up for serological evaluation of diseases included in the national immunization programme to which priority had been given.

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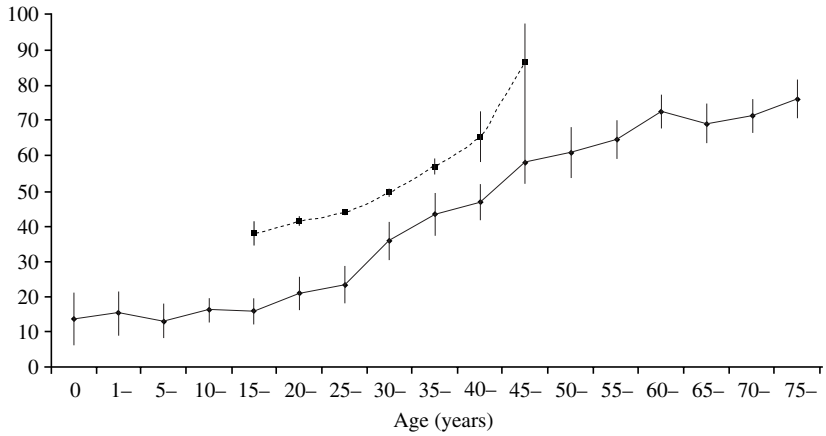

Fig. Age-specific prevalence of *Toxoplasma gondii* antibodies in the Pienter project (1995–1996; $n=7521$; –◆–) and in the TIP study (1987–1989; $n=27\,967$; ---■---) [9].

Statistical analysis

Seroprevalences within each municipality were weighted by the proportion of the age group in the population. To produce national estimates, the weighted frequencies were averaged over the 40 municipalities [12]. Information on all participants and non-participants was available for age sex, marital status, country of nationality, degree of urbanization, region and whether reminded by telephone or by mail [14]. The effect of differential probabilities of response for these variables on the national estimate amounted to less than 1 S.E. and was therefore ignored.

Logistic regression analysis was used to determine whether any of the following variables were independent predictors of seropositivity for *Toxoplasma gondii*, after adjustment for age group: marital status, country of birth, educational level, geographical region, urbanization, gardening, contact with animals by virtue of one's profession in the past 5 years, keeping a cat in the last 5 years, keeping a dog in the past 5 years and keeping a rabbit, hamster or guinea pig in the past 5 years.

The educational levels of those aged $\geqslant$17 years and of one of the parents for those aged $<$17 years, were classified as 'low' (primary school, lower vocational or lower general secondary education), 'intermediate' (intermediate vocational or intermediate general secondary and higher general secondary education) and 'high' (higher vocational secondary education and university education). The following categories for level of urbanization were used: 'very high' (>2500 addresses/km^2), 'high' (1500–2500), 'moderate' (1000–1500), 'low' (500–1000), and 'none' (<500). The geographical regions were based on the Dutch provinces: 'Central' (Utrecht and Gelderland), 'Southeast' (North Brabant and Limburg), 'Northwest' (North Holland and Flevoland), 'Southwest' (Zeeland and South Holland) and 'Northeast' (Groningen, Drente, Overijssel and Friesland). Marital status was categorized as 'married, living together or unmarried' *vs.* 'divorced or widowed', country of birth as 'The Netherlands' *vs.* 'other', gardening as 'less than 5 h a week' *vs.* 'more than 5 h a week'. The remaining variables were classified as 'yes' or 'no'. Statistical analyses were performed with SAS 8.1 (SAS Inc.).

In order to be able to compare a group with higher risk for Toxoplasma seropositivity with a group with lower risk we studied predictors of seropositivity for *Toxoplasma gondii* antibodies in two age groups (<1–19 years and 20–79 years) separately.

RESULTS

Prevalence of *Toxoplasma gondii* antibodies

The overall seroprevalence in the Pienter study amounted to 40·5% (95% confidence interval 37·5–43·4). The seroprevalence varied with age (Fig.). It was less than 17% among those <20 years, but increased from 21% in the 20–24 years age group to 47% among the 40–44 years age group to at least 70% for those aged 60–64 years or over. The increase was steepest among the 25–45 years group compared to both younger and older age groups. No differences were found between men (39·3%, 95% CI 35·7–42·9) and women (40·9%, 95% CI 37·9–43·8). Also for women of reproductive age (15–49 years) there was

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Table. *Prevalence of specific antibodies to* Toxoplasma gondii *(%) and odds ratios (OR) (multivariate logistic regression analysis) for seropositivity for* Toxoplasma gondii *in participants <1–19 years and 20–79 years respectively*

	Age group: <1–19 years			Age group: 20–79 years		
	n = 2311	%	Adj. OR[a]	*n* = 5210	%	Adj. OR[b]
Marital status						
Married/Living together/Unmarried	2311	—		4651	35·9 (30·1–41·7)	1·0
Divorced/Widowed	—	—		559	48·4 (44·9–51·9)	**1·4** (1·1–1·7)
Country of birth						
The Netherlands	2199	14·2 (10·3–18·0)	1·0	4913	48·6 (45·0–52·3)	1·0
Other	112	23·8 (13·8–33·8)	**2·1** (1·3–3·4)	297	51·4 (47·7–55·0)	1·2 (0·9–1·5)
Educational level						
High	639	13·8 (8·9–18·7)	1·0	890	42·3 (38·4–46·2)	1·0
Medium	819	13·6 (9·3–17·9)	1·0 (0·7–1·3)	1295	45·5 (40·6–50·3)	1·1 (0·9–1·4)
Low	802	15·7 (11·0–20·3)	1·0 (0·8–1·4)	2982	52·5 (48·2–56·8)	**1·4** (1·2–1·7)
Region						
Southeast	476	19·5 (6·8–32·1)	1·0	1087	36·4 (27·5–45·4)	1·0
Central	464	17·9 (6·2–29·5)	0·9 (0·6–1·2)	1037	47·8 (41·9–53·7)	**1·8** (1·5–2·2)
Northwest	462	21·9 (13·1–30·7)	1·1 (0·8–1·6)	1016	58·8 (51·6–66·1)	**3·1** (2·5–3·8)
Northeast	470	6·7 (3·2–10·2)	**0·3** (0·2–0·5)	1062	49·2 (40·6–57·8)	**1·8** (1·5–2·1)
Southwest	439	8·9 (3·6–14·3)	**0·4** (0·2–0·6)	1008	52·3 (48·1–56·5)	**1·9** (1·6–2·3)
Urbanization						
High	262	16·1 (0·8–31·3)	1·0	571	43·5 (28·4–58·7)	1·0
Very high	278	19·8 (6·4–33·2)	**1·6** (1·0–2·5)	650	45·7 (37·1–54·2)	**1·2** (1·0–1·6)
Intermediate	589	16·8 (9·5–24·1)	1·1 (0·8–1·7)	1372	53·6 (45·3–61·9)	**1·4** (1·2–1·8)
Low	494	12·7 (0·5–24·9)	1·0 (0·6–1·6)	1081	44·6 (38·2–51·0)	1·2 (0·9–1·5)
No	688	11·9 (3·8–19·9)	0·9 (0·6–1·4)	1536	52·0 (43·8–60·2)	**1·3** (1·1–1·6)
Gardening						
Less than 5 h a week	2160	**14·4** (10·7–18·2)	1·0	4489	48·2 (44·7–51·7)	1·0
At least 5 h a week	151	19·8 (10·9–28·6)	**1·5** (1·0–2·3)	721	57·3 (51·2–63·4)	**1·2** (1·0–1·4)
Professional contact with animals in the past 5 years						
No	2294	14·9 (11·1–18·8)	1·0	4930	48·4 (44·9–51·9)	1·0
Yes	17	19·2 (0·0–42·4)	1·4 (0·4–5·1)	280	52·2 (45·3–59·0)	**1·5** (1·2–2·0)
Keeping a cat in the past 5 years						
No	1546	14·9 (10·6–19·3)	1·0	3830	48·5 (44·6–52·4)	1·0
Yes	765	13·5 (9·7–17·3)	0·9 (0·7–1·2)	1380	50·2 (46·5–53·8)	**1·2** (1·1–1·4)
Keeping a dog in the past 5 years						
No	1547	14·8 (10·7–18·9)	1·0	3725	48·2 (44·5–51·9)	1·0
Yes	764	16·7 (11·6–21·9)	1·0 (0·8–1·3)	1495	50·4 (45·2–55·5)	1·0 (0·9–1·1)
Keeping a rabbit, hamster or guinea pig in the past 5 years						
No	1507	15·9 (11·9–19·9)	1·0		49·5 (45·7–53·2)	1·0
Yes	804	13·4 (8·9–18·0)	0·8 (0·6–1·0)	748	41·1 (35·6–46·6)	**0·9** (0·7–1·0)

[a] Odds ratio adjusted for age groups 0 and 1–19 years and all variables included in the table.
[b] Odds ratio adjusted for age groups 20–29, 30–44, 45–59 and 60–79 years and all variables included in the table.
The 95% confidence interval is given within parentheses. The OR is considered to be significant if 1 is not included in the 95% confidence interval. For example an OR of 1·2 with an interval of (1·1–1·4) means a significantly increased risk; an OR of 0·4 with an interval of (0·2–0·6) means a significantly decreased risk.

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no difference from men (35·2%, 95% CI 32·9–38·6 *vs.* 34·7, 95% CI 30·2–39·2). The seroprevalence in the Southwest region was 38·7 (95% CI 34·6–42·7) for the 15–49 years age group, increasing from 11·2% (95% CI 2·3–20·2) for the 15–19 years group to 65·2% (95% CI 50·4–80·1) in the 45–49 years group.

In the TIP study the overall seroprevalence was 45·8% (95% CI 45·2–46·3) and increased with age from 38·2% (95% CI 34·8–41·7) in the 15–19 years age group to 86·7% (95% CI 62·5–97·7%) in women aged $\geqslant$45 years.

The national estimate of seroprevalence of the Pienter study decreased by more than 10% to 35·2% (95% CI 32·9–38·6), in comparison with the seroprevalence in the TIP study (45·8%) (see Fig.).

Predictors of seropositivity for *Toxoplasma gondii* antibodies

The Table shows that by multivariate logistic regression analysis differences were found in independent predictors for *Toxoplasma gondii* seropositivity between participants aged <1–19 years and those aged 20–79 years.

For those <20 years of age, being born outside The Netherlands, very high urbanization level, living in the Southeast, Central or Northwest region of The Netherlands and gardening for at least 5 h a week were independent predictors of *Toxoplasma gondii* seropositivity. Divorced or widowed marital status, low educational level, living in the Central, Northwest, Northeast or Southwest region of The Netherlands, very high, intermediate and no urbanization level, gardening for at least 5 h a week, professional contact with animals in the past 5 years and keeping a cat in the past 5 years, were independent predictors for participants aged 20–79 years.

In the youngest age group the effect of marital status could not be studied since almost all participants were unmarried. Furthermore the odds ratio (OR) for professional contact with animals in this age group was similar to the OR for the 20–79 years age group but probably resulted from low numbers (the 95% confidence interval included unity).

DISCUSSION

Our study showed that approximately 40% of the Dutch population aged <1–79 years have been infected with *Toxoplasma gondii* during their life. Toxoplasma seroprevalence in The Netherlands increases with age from 1% in <1-year-olds to 79% in those aged 75–79 years. The steepest rise – from 20 to 50% – was found in the 25–44 years age group, i.e. a subset of the reproductive age groups. However, the seroprevalence among women of reproductive age (15–49 years) in our study (1995–1996) was 10% lower (35%) by comparison with data from the TIP study, among women of reproductive age, approximately 8 years earlier (46% for 1987–1988) (see Fig.) [11]. Longitudinal interpretation of cross-sectional surveys can be problematic because of the danger of overlooking cohort effects. The slope of the 1987–1988 and 1995–1996 curves are similar and it could be concluded that the risk of acquiring infection in this age range has not changed. However, moving the 1988 curve about 8 years towards the right, in effect comparing similar birth cohorts, points to a decreased risk of infection in the past 8 years. Since the seroprevalence among men and women in our study (1995–1996) was similar, this possible change probably does not reflect an effect of primary preventive measures among women of childbearing age. It might reflect a decrease in force of infection in raw meat or livestock. A decline in seroprevalence over the past three decades has been reported in other countries. In our study we found that living in the Northwest of the country, professional contact with animals, e.g. working in a slaughterhouse or farming, living in a moderately urbanized area, being divorced or widowed, being born outside The Netherlands, gardening more than 5 h per week and owning a cat were independently associated with Toxoplasma seropositivity. However, the ORs were small.

We have no explanation yet for the regional differences nor for the effect of urbanization. The Northwest part of The Netherlands, with the highest risk for Toxoplasma seropositivity, is located near the sea and might be considered climatologically different, wetter and milder. However, The Netherlands is relatively small geographically. There are differences in consumption of specific regional food items in the different parts of The Netherlands, which may include undercooked meat. Unfortunately, in our study no information was collected on the consumption of undercooked meat since the main focus of the questionnaire was on vaccine-preventable diseases as stated in the Introduction. In a population-based case-control study in The Netherlands, conducted in 1999 to study the incidence

of gastroenteritis and in which we were able to ask detailed questions about eating habits we found only a small percentage of cases and controls ate undercooked meat (less than 2·5% undercooked beef, less than 2·2% undercooked pork and less than 0·4% undercooked chicken). There is a relation with age and eating undercooked meat: small children and adolescents eat less than adults (1·6, 0·6 and 10·7% respectively) but there is no clear relation with urbanization or region (unpublished data, SENSOR study [15]). We do not expect that eating of raw or undercooked meat will have had a major confounding effect on the other risk factors. In the European multicentre study, eating raw sausages more than once a week, eating raw or undercooked beef and lamb and tasting meat while cooking were the most important risk factors [7]. In The Netherlands lamb is not frequently consumed, although increasingly popular, but beef and pork are. Data collected in the 1980s showed that the seroprevalence in fattening pigs was 1·8% and in fattening calves 1·2% [16–18]. These studies have not been repeated more recently. However, in the past three decades several European countries reported a decrease in prevalence, especially in fattening pigs [19].

Interestingly, there is a difference in the effect of region and urbanization as well as for other independent risk factors for Toxoplasma seropositivity in the <20 years and >20 years age groups. In the older age group the ORs for those living in the Northwest (OR 3·1) or Southwest (OR 1·9) region are higher compared with the reference region, the Southeast. The OR for living in a town with high urbanization degree (OR 1·0) is lower compared to the other categories (OR 1·2–1·4). In contrast, in the younger age group the risk in the Southwest is one of the lowest (OR 0·4), while for living in a town with a very high level of urbanization it is high (OR 1·6) compared to the other categories (OR 0·9–1·1). This suggests a change in (yet unknown) risk factors associated with region and urbanization in the last decades. However, interpretation is difficult, even more so, as residence at the moment of our study does not necessarily reflect the original place of birth and childhood for adults.

Only in the younger age group is country of birth outside The Netherlands independently associated with Toxoplasma seropositivity. This might be due to an increasing difference between seroprevalence in The Netherlands and other countries in the last decade. Most people born outside The Netherlands originate from the Mediterranean area (Turkey, Morocco) or South America (Surinam, Antilles). The decrease in seroprevalence in The Netherlands may only have occurred to a lesser extent in these countries. In Turkey seroprevalence varies between 27 and 80% in women of childbearing age. Seroprevalence is higher in countries in South America, including Argentine, Cuba, Venezuela and Brazil (51–72%) [19].

Only for the 20–79 years age group was low educational level an independent predictor for seropositivity. This might be that as a result of lower hygienic standards among the lower social economic classes in former times, the chance of *Toxoplasma gondii* infection in childhood could have been higher than among the higher economic classes. Nowadays no difference is observed between the classes. We cannot explain the observation of an association of an increased risk for Toxoplasma seropositivy and being divorced or widowed. Since the ORs are adjusted for age, owning a cat and gardening, other factors like changes in hygiene, or food habits might be an explanation.

Keeping a cat during the past 5 years is only weakly associated with Toxoplasma seropositivity and only for those aged $\geqslant 20$ years, while no association was found for keeping a dog, rabbit, hamster or guinea pig. Others also reported that owning a cat gave a slight increase in Toxoplasma infection [8]. The absence of a risk for owning a cat among the younger age groups is difficult to interpret since we have neither information on the total number of years of cat contact in our study population nor on the (changing) prevalence of toxoplasmosis in cats. Techniques of caring for cats may have changed over the years. The practice of allowing cats to defaecate in a place with strongly absorbent granules can influence the infectivity of the oocysts, since they are vulnerable to desiccation. The increased OR in both age groups for those who work in the garden for at least 5 h a week shows that exposure to oocytes in the environment is a risk factor. Our study showed an increased risk for those with professional contact with animals in the past 5 years, for example farmers. This effect shows the risk of infection by ingestion of oocysts.

These results confirm that the cat itself is not a risk factor but that the oocysts excreted by the cat in the environment are [19, 20].

In conclusion, our study shows that postnatally acquired toxoplasmosis still occurs regularly in

The Netherlands. To obtain more insight into risk factors for community-acquired infection studies directed specifically to *Toxoplasma gondii* and with collection of more detailed information on potential risk factors, i.e. raw meat consumption and other eating habits, would be useful, preferably obtained, although costly, by a longitudinal study. This would overcome the disadvantage of cross-sectional studies, in which the outcome variable, seropositivity, is a cumulative measure of all past exposures during life.

Although seroprevalence decreased among women of reproductive age in the last decade, the steepest rise in seroprevalence still occurred among this subset aged 25–44 years. A similar rise was also seen for men. It shows that women are still at considerable risk of being infected during pregnancy and underscores the importance of informing women of reproductive age of the message: 'Don't eat raw or undercooked meat, clean all vegetables, wear gloves during gardening and don't clean cat boxes yourself!'

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