

Seroprevalence of Human Toxocariasis in Denmark[∇]

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The seroprevalence of *Toxocara* in the Danish population was assessed from 3,247 sera from individuals originally screened for toxoplasmosis. Of 87 enzyme-linked immunosorbent assay-positive sera, 79 were confirmed by Western blotting, yielding a crude seroprevalence of 2.4%. This indicates that the seroprevalence of toxocariasis in Denmark is low compared to those in other European countries.

Human toxocariasis is a parasitic zoonosis caused primarily by *Toxocara canis* and *Toxocara cati*, which are intestinal roundworms of canids and felids of worldwide occurrence (2, 5, 13, 14). Transmission is fecal-oral, and infections in human hosts can occur upon accidental ingestion of embryonated eggs as a cause of geophagia, poor hygiene, contact with dogs and cats, and ingestion of raw vegetables (2, 13, 16). Depending on the organs affected, clinical manifestations due to the aberrant migration of second-stage larvae include visceral larva migrans, ocular larva migrans, and neurologic and covert toxocariasis (2, 4, 5). Serodiagnosis of toxocariasis is usually performed by enzyme-linked immunosorbent assay (ELISA) screening using *Toxocara* excretory-secretory antigens followed by a confirmatory analysis—for instance, Western blotting (WB) (14). However, despite the fact that such reliable immunodiagnostic methods are currently readily available for serological diagnosis of toxocariasis, few seroepidemiological studies have been carried out to elucidate the background prevalence of toxocariasis (14). The aim of this study was to determine the seroprevalence of human toxocariasis in the Danish population.

In 2001, a total of 3,247 serum samples randomly chosen from panels of sera from Danish citizens originally screened for *Toxoplasma gondii* were anonymously submitted to *Toxocara* antibody testing. Of all samples, 2,551 were from presumably healthy, women 20 to 39 years old, the vast majority of whom had been screened for toxoplasmosis during pregnancy. Gender and age information was available for all *Toxocara*-positive samples and 840 negative samples (in total, 28% of all samples), giving a representative distribution for age and sex. A projected total number of *Toxocara*-negative samples by sex and age was calculated from this distribution (multiplying all original numbers by 3.77), and age-specific prevalence rates were subsequently calculated. No statistical data analysis was performed due to this projection of records.

Serum immunoglobulin G antibodies were detected using an

ELISA kit for the serological diagnosis of human toxocariasis (Bordier Affinity Products SA, Crisser, Switzerland) according to the recommendations of the manufacturer. Hence, sera were diluted 1:201. The optical density range for the weak-positive control in all ELISAs performed was between 0.574 and 1.393, and in order not to exclude possibly positive sera, the cutoff was set to 0.500 for WB confirmation. Excretory-secretory antigen for WB was prepared from *T. canis* larvae by in vitro cultivation and concentration using Amicon ultrafiltration membranes (nominal molecular weight limit, 10,000) (15). Antigen quality control was performed by ELISA using well-defined human serum samples of patients with different helminthic diseases. The specificity of the ELISA was assessed by WB, where two low-molecular-mass bands of 30 to 32 kDa were considered *Toxocara* specific (11, 14).

Of 3,247 serum samples, 87 were positive for *Toxocara* by initial ELISA screening, 79 of which were positive by confirmatory WB analysis (Table 1). Hence, the overall prevalence among study individuals was 2.4%. No WB negatives were detected among samples with an ELISA cutoff above 0.800. Of the 79 *Toxocara*-positive individuals, 21 (27%) were seropositive for *Toxoplasma*.

Based on data pertaining to the males, it appears that the seroprevalence was independent of age (Table 1). This is consistent with an expectation of antibody reactivity decline over time, as seen in both patients with echinococcosis and nonviable cysts, who lost antibodies after 4 years (17), and patients with schistosomiasis, who were antibody negative on average 10 years after treatment (3). The majority of the samples (77.4%) in the present study represented sera from presumed healthy women 20 to 39 years of age, who had submitted a blood sample for pregnancy-related routine characterization of *Toxoplasma* serostatus. Therefore, the reported data on *Toxocara* seropositivity in this population segment are likely to more or less reflect the overall background seroprevalence in Denmark. Sera from males and females in other age groups (Table 1) more likely represented samples from symptomatic individuals. Not surprisingly, these groups of individuals taken together had an approximately fourfold-higher prevalence of toxocariasis than the pregnant women, which does not necessarily indicate a higher prevalence of disease but does indicate

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TABLE 1. Seroprevalence of human toxocariasis in Denmark determined by ELISA and WB in asymptomatic and symptomatic individuals^a

Age (yr)	Males			Females			Total		
	<i>n</i>	No. positive	Prevalence (%)	<i>n</i>	No. positive	Prevalence (%)	<i>n</i>	No. positive	Prevalence (%)
0–9	87	8	9.2	62	2	3.2	149	10	6.7
10–19	94	3	3.2	91	8	8.8	185	11	5.9
20–29	46	4	8.7	1,368	18	1.3	1,414	22	1.6
30–39	61	1	1.6	1,183	21	1.8	1,244	22	1.8
≤40	102	4	3.9	153	10	6.5	255	14	5.5
Total	390	20	5.1	2,857	59	2.1	3,247	79	2.4
Mean ± SD			5.3 ± 3.4			4.3 ± 3.2			4.3 ± 2.4

^a The asymptomatic individuals represent females 20 to 39 years of age, and the symptomatic individuals represent other groups.

higher exposure and risk. The assumption that the women aged 20 to 39 years included in the study represent the general background population (i.e., both males and females) is primarily based on the observation that a gender-related difference in prevalence among the other age groups could not be detected (Table 1).

To our knowledge, this is the first study on the *Toxocara* seroprevalence in Denmark. Limited data on the background seroprevalence in European countries are available. A prevalence of 6.6% was found among 201 healthy Italian individuals (12). Havasiová et al. (6) reported a prevalence of 13.65% among healthy Slovakian blood donors, whereas Stürchler et al. (16) and Jacquier et al. (7) demonstrated prevalences of 5% and 4% among Swiss blood donors, respectively. Data from the analysis of Swedish sera from 323 healthy individuals and 175 patients with clinical signs and symptoms, such as eosinophilia and ocular, pulmonary, hepatic, or neurological disorders revealed seroprevalences of 7% and 25%, respectively (10). The present data corroborate a significant difference in seroprevalence between the background population and patient segments. However, the background prevalence in the present study, based on women between 20 and 39 years of age, was 1 to 2% and was not nearly as high as in the Swedish study, which might partly be explained by a difference in the dilution of sera used in the ELISA screening and the fact that WB was not used in the Swedish study. It is also likely that samples from asymptomatic individuals used in the Swedish study represented another type of study material that is not immediately comparable with the present material.

Data from an American study have shown that persons infected with *Toxocara* are more likely to be infected with *Toxoplasma* (8). The seropositivity of *Toxoplasma* among the *Toxocara*-positive individuals in the present study was similar to the general seroprevalence of *Toxoplasma* among pregnant women in Denmark reported by Lebech et al. (9). It is possible that the majority of European *Toxoplasma* infections are contracted upon the ingestion of raw or undercooked *Toxoplasma*-infected meat, whereas the role of soil-transmitted and waterborne toxoplasmosis in the United States may be more important (1).

Conclusively, these data reflect that the background seroprevalence of toxocariasis in Denmark is low compared to those in other European countries. However, a significant difference in seroprevalence exists between healthy, pregnant

women and patients contacting their general practitioners for reasons other than pregnancy, which suggests that symptomatic cases of toxocariasis are not rare in Denmark.

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