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Epidemiologic approach to human toxocariasis in western France

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Abstract *Toxocara canis* is a common parasite in puppies. The danger to human health has not been properly established. We estimated the current incidence of this pathogen in two western districts of France, Loire-Atlantique and Maine et Loire. Blood samples from 1836 eosino-philic patients were collected and tested by an enzyme-linked immunosorbent assay (ELISA) excretory-secretory *Toxocara* antigen test. We obtained positive results in 22% of the cases and highly positive results in 7%. The ELISA data seemed to be age-dependent, with older patients having more positive results ($P < 0.0001$). The interlaboratory distribution of positive test results was statistically significantly different ($P < 0.0001$), suggesting regional sources. The main clinical expressions of toxocariasis were: asthenia, gastric pain, and pulmonary disease. Individual and collective surveys of this zoonotic disease need to be carried out.

Introduction

In temperate climates, human toxocariasis is probably the most common zoonotic helminthiasis. Beaver et al. (1952) described the first case of visceral larva migrans (VLM) due to *Toxocara canis* larvae. The genus *Toxocara* includes *T. canis* and *T. cati*, dog and cat roundworms,

respectively. The definitive host is infected by ingestion of embryonated eggs. After maturation and migration, stage 2 larvae (L₂) are widely distributed through the systemic circulation (Gillespie 1988). This situation is far from anecdotal, since there are more than 10 million domestic dogs and 7.5 million domestic cats in France (Baverel 1993). A bibliographic review of urban and rural infection in France and in Europe has been conducted by Barriga (1988), who reported a wide variation in soil contamination levels (ranging from 1.3% in Sweden to 77.8% in France). Petithory and Ardoin (1990) have emphasized that better detection of canine helminth eggs would allow us to pinpoint those actually due to *T. canis*, but one should not underestimate the importance of this potential environmental pathogen. The clinical symptoms of human toxocariasis presently seen include the following:

1. The ocular form, or ocular larva migrans, is uncommon and almost always isolated (Petithory et al. 1990; Benzacken et al. 1992; Gautier 1992). It was first described by Wilder (1950). Clinical observations include uveitis, retinal granuloma or chronic endophthalmitis. Visual acuity can also be impaired (Gueglio et al. 1991).

2. The VLM syndrome described by Beaver et al. (1952), is usually associated with a history of pica; the symptoms are hepatomegaly, fever, coughing and wheezing, and central nervous system involvement with persistent eosinophilia.

3. The mild syndrome has been recently been described (Magnaval et al. 1983; Taylor et al. 1988). The symptomatology is nonspecific, consisting of chronic weakness associated with moderate but persistent eosinophilia. Both children and adults are affected and this seems to be the most frequently occurring clinical expression (Gueglio et al. 1991; Loiseau 1991).

For the diagnosis of toxocariasis it is essential to have both epidemiologic and biologic data. The choice of therapy remains difficult. Experiments involving the egg maturation cycle of *T. canis* have been carried out by de Savigny (1975). This allowed the development of highly sensitive and specific serologic tests using a *Toxocara*

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excretory-secretory antigen. Due to common antigenic determinants between different tropical nematodes and *Toxocara* spp., serologic interpretation can be difficult if the patient has lived in tropical areas. Therefore, toxocariasis immunodiagnosis is mostly used for patients in temperate zones, especially in cases of eosinophilia. Our aim was to measure the incidence of toxocariasis in rural populations for two districts in western France: Loire Atlantique and Maine et Loire.

Materials and methods

Blood sample from patients with eosinophilia greater than 500 cells/mm³ were included in the study. The blood samples were contributed by private and institutional public health laboratories in both districts, including 8 of 60 laboratories in Loire Atlantique and 6 of 39 laboratories in Maine et Loire. Any blood sample with eosinophilia, drawn from patients living in the two study zones, was forwarded to us. A control population from non-eosinophilic patients was evaluated in Loire Atlantique and comprised 10 random donors from each participating laboratory (79 samples; 1 sample missing). The enzyme-linked immunosorbent assay (ELISA) test using *Toxocara canis* L₂ larval excretory-secretory antigen was performed according to the manufacturer's instructions. (Bordier Affinity Products, 1013 Crissier, Lausanne, Switzerland). The results were graded qualitatively as follows: 0, negative; 1, positive; 2, highly positive. If a positive or highly positive result was obtained, an epidemiologic clinical questionnaire was sent to the patient's physician via the participating laboratory. ELISA specificity/sensitivity was evaluated on 100 randomly chosen samples by a Western blot *Toxocara* test (Dr J.F. Magnaval, Toulouse, France), by an ELISA trichinosis test (Dr C. Aznar, Institut Pasteur, Paris, France), and by a latex ascariasis test (coelomic antigen; Prof. R. Robert, Angers, France). Statistical analysis was carried out with EPI INFO 5 software.

Results

From 1 February 1992 through 31 July 1992, 1836 blood samples were collected from eosinophilic patients as follows: 912 samples from Loire Atlantique (49.7%) and 924 samples from Maine et Loire (50.3%). The mean age of the population was 47.6 years (range, 1–98 years) and the ratio of women to men was 1.13.

General results

The ELISA values are shown in Fig. 1; 22% were positive, with 7% being highly significant for toxocariasis pathology. Regarding sex differences, we noted that younger men (46 ± 26 years old) versus older men (51 ± 27 years old, $P = 0.001$) were more frequently positive in the serologic test ($P = 0.009$; Table 1). The mean eosinophil results were not significantly different with regard to sex ($P > 0.05$) or ELISA values ($P > 0.05$; Tables 2, 3). In contrast, *Toxocara* antibody titers increased among older subjects ($P < 0.0001$; for ELISA scores of 0, the mean age was 46 years, whereas for ELISA: 1 or 2, the mean age was 52 years. In the Maine et Loire district, seropositive tests were more frequent ($P < 0.01$). Both districts

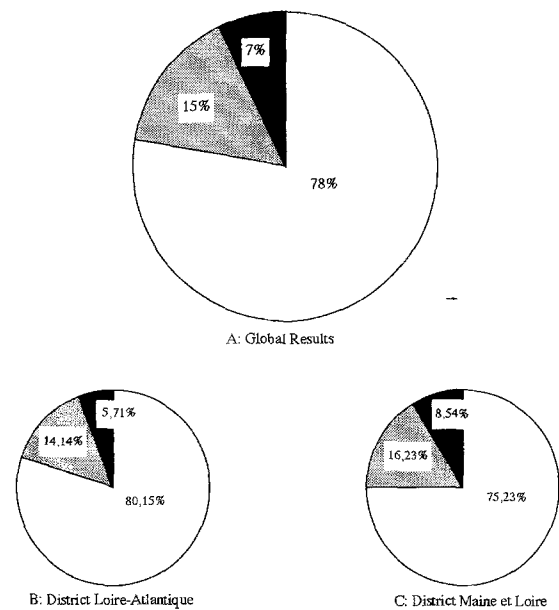


Fig. 1A–C General results: global results (A) are shown above, with each study district (B, C) being shown below: (ELISA 0, negative; ELISA 1, positive; ELISA 2, highly positive)

Table 1 Correlation between patients' sex and ELISA values: a positive ELISA value (1 positive, 2 highly positive) is more frequent in the male population ($\chi^2 = 6.8$)

n=1801 (nonspecified: 35)	Female	Male
ELISA 0	686	714
ELISA 1 or 2	167	234

Table 2 Comparison of mean eosinophil values by patients' sex: no significant difference is seen.

	Female	Male
Mean eosinophil value/mm ³	826 ± 450 n = 877	817 ± 325 n = 993

Table 3 Comparison of mean eosinophil values by ELISA value: no significant difference is seen.

	ELISA 0	ELISA 1 or 2
Mean eosinophil value/mm ³	818 ± 436 n = 1409	825 ± 392 n = 406

presented similar populations according to the age distribution ($P > 0.05$). Concerning individual laboratories, there were significant differences in age distribution ($P < 0.0001$) and mean eosinophil values ($P = 0.001$). These differences could have been due to the use of different laboratory methods i.e., automated versus manual blood counts. The distribution of positive tests according to each center was statistically significantly different ($P < 0.0001$), and allows for the identification of the sources of toxocariasis.

Table 4 Western blot (WB) and ELISA *Toxocara* results: the WB *Toxocara* assay is more sensitive than the ELISA *Toxocara* test. (0 negative, 1 positive, 2 highly positive) WB incomplete: <7 bands

Totals WB <i>Toxocara</i>	ELISA <i>Toxocara</i>			
	0	1	2	
Negative	33	6	0	39
Incomplete	1	0	1	2
Full pattern	19	22	17	58
Totals	53	28	18	99

Table 5 ELISA versus WB *Toxocara* characteristics: eosinophilia increases ELISA score-2 sensitivity with a 100% predicted positive value

	ELISA 1 or 2	ELISA 2
Sensitivity	65%	28.3% (45% for eosinophilia)
Specificity	86.8%	100%
Predicted positive value	88%	100%

ELISA test characteristics

In all 100 randomly chosen samples among a total of 1915 samples (1836 patients and 79 controls) were tested (a) by a Western blot (WB) *Toxocara* test (data shown in Tables 4, 5); (b) by a trichinosis ELISA test whereby one serum tested positive but all biologic toxocariasis tests remained negative; (c) by an ascaridiasis latex test,

whereby one serum tested positive. Both the ELISA test for *Toxocara* and that for trichinosis remained negative, as did the W B *Toxocara* test. Therefore, we did not see any cross reactivity with trichinosis or ascariasis. The ELISA test gives a good specificity, though it is less sensitive than Western Blotting in toxocariasis screening.

Clinical features

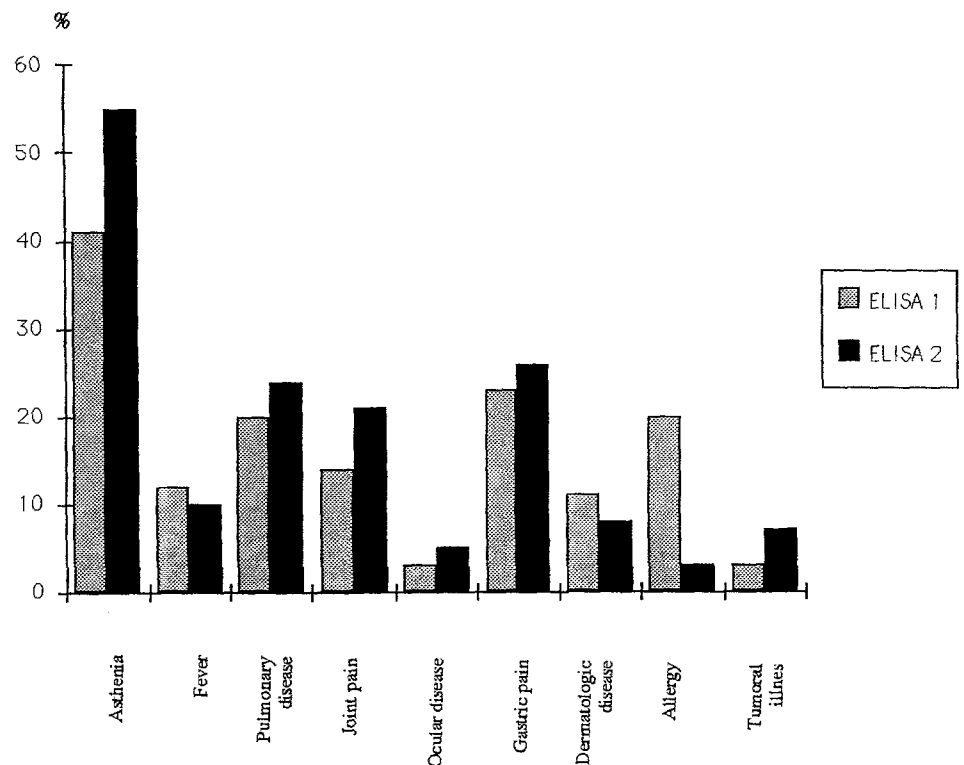
From 423 patients with positive ELISA *Toxocara* results (scores of 1 or 2), we obtained 280 clinical questionnaires. No diagnostic score could be defined. However, according to the histograms (Fig 2), we observed some clinical data more frequently: asthenia, gastric pain, and pulmonary disease.

Discussion

Study population

Before analyzing our data, we compared our study population with the results of the last population census for the two districts (Fig 3), which was conducted in 1990. Children younger than 10 years of age were well represented. We observed a higher incidence in patients older than 60 years, probably due to an increase in medical problems in elderly patients. Regarding the younger age groups, we did not find any difference between the two districts, in which more than 50% of the residents were younger than 40 years.

Fig. 2 Clinical features observed in toxocariasis among 423 patients with ELISA scores of 1 or 2 (1 positive, 2 highly positive)



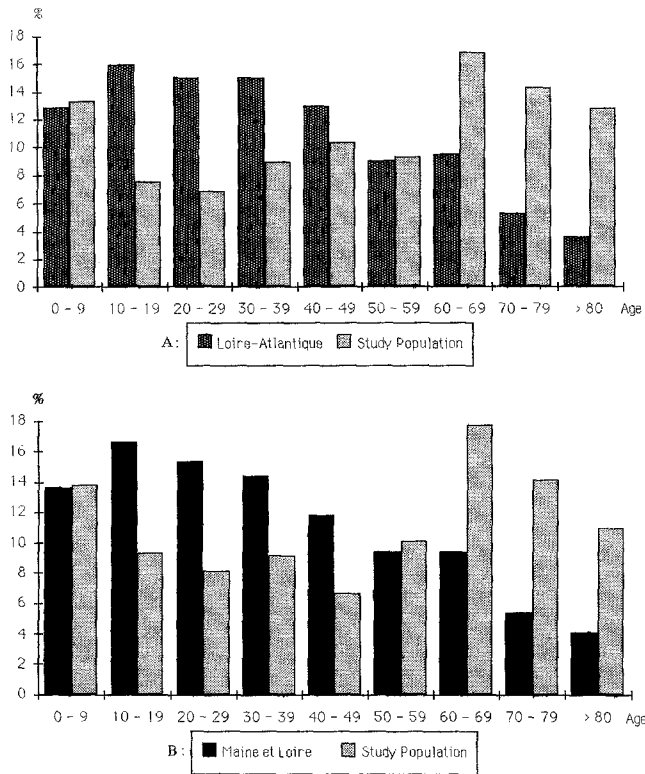


Fig. 3A,B Comparison of age: study population versus general population (*census figures 1990*): **A** Comparison regarding Loire-Atlantique district. **B** Comparison regarding Maine et Loire district

Regional aspect of toxocariasis

Since the ELISA screening test found that 22% of the eosinophilic population from the two districts were carriers of antibodies (7% of whom had high levels), several observations can be made. Eosinophilia is a biologic sign of larval migration in helminthiasis. Toxocariasis is the typical cause of VLM syndrome. Nevertheless, we could not establish significant differences between the mean eosinophil levels of these patients and their ELISA values. In a study performed in Ireland in suspected toxocariasis patients, 27% had normal eosinophil counts (Taylor et al. 1988). As long as eosinophilia remains an indication for potential larval pathogenesis, this biologic abnormality does not influence the serologic response. Adults are more often afflicted with toxocariasis than children (Loiseau 1991). Men are more frequently affected than women. Professional activities such as gardening and hunting with dogs also represent cumulative risks. The more often the subject is exposed to possible contamination, the more likely are the chances of contracting the disease. We observed four foci of high incidence. Two of them are located in the north of Maine et Loire district, a largely rural agricultural region. The inhabitants, mostly farmers, possess gardens and domestic animals and indulge in outdoor or soil-related activities. The source on the Atlantic coast at Pornic is difficult to explain, except for the abundance of stray cats. However,

the high incidence found in Briere, where samples were collected by the Pontchateau laboratory, involves the marshlands which contain people with a self-sufficient life style. The Briere population¹ is not known for its attention to health care; local residents suffer from rheumatic diseases and asthmatic-type respiratory problems. This symptomatology is compatible with the eventual expression of *Toxocara* disease.

Correlation with other incidence studies

Many European toxocariasis studies have been performed using similar biologic tests (Elisa); the diagnosis of toxocariasis is possible only with immunodiagnosis. In Italy, Scaglia et al. (1988) reported a 7.2% prevalence among apparently healthy blood donors. Di Fiore et al. (1989) noted a positive correlation between patients' behavior and positive *Toxocara* serological tests. Genchi et al. (1990) found that the prevalence of the disease increased with age and type of professional activity (soil-related or outdoor profession, e.g., tire repairers, gasoline station attendants, garbage collectors, gardeners, masons, and carpenters). Studies conducted in Spain by Portus et al. (1989) revealed a serologic prevalence of 14% among adult eosinophilic patients without etiology, whereas Cauchie et al. (1990) found a prevalence of more than 5% in Belgium. *Toxocara* is therefore not a rare pathogen in economically developed countries and affects adults more often than children. The clinical and serologic expression is strongly dependent on risk factors accumulated over time (Kimming et al. 1991). Except for peculiar nutritional behavior such as eating raw giblets (Nagakura et al. 1989) or – as related to earlier prophylactic therapy for ulcers – eating raw snails (Romeu et al. 1991), the accidental ingestion of infective *Toxocara* larvae occurs principally in cat and dog owners, gardeners, and professionals in contact with domestic animals or cattle. Pica remains a way of transmission in children, occurring, soon after they have begun to walk.

Clinical study

This study revealed a high serologic incidence of *Toxocara* ssp, with 22% of the population being antibody carriers. Analysis of clinical questionnaires confirmed a positive serologic test for patients with an ELISA score of 2 (7% of the study population.). No diagnostic score could be defined. However, in *Toxocara* disease we should retain the three symptoms asthenia, gastric pain, and pulmonary symptoms in association with an ELISA score of 2 and eosinophilia. Although pain, especially joint pain, are frequently reported, the etiology observed seemed to be more rheumatologic than parasitologic. Nevertheless, *Toxocara* rheumatologic aspects have been described (Gueglio et al. 1993 Dromer et al. 1994) and

¹An anthropology study of this population has confirmed its homogeneity (Prof. R. Robert, personal communication)

involve very precise criteria (Doury et al. 1977). Cutaneous disorders such as rash, urticaria, and pruritus can also be a signal of the presence of *Toxocara* larvae in the host. In conjunction with gastric pain, these symptoms express the disease in dogs. In humans, these symptoms accompanied by eosinophilia should evoke questions of parasitosis as well as allergic phenomena. In our study we did not note any ocular manifestation. *Ocular larva migrans* remains uncommon, with less than 2000 cases being reported worldwide (Petithory et al. 1990) but not exceptional (Leewan et al. 1991; Gautier 1992; Gueglio et al. 1993). As some investigators have noted (Magnaval 1987; Magnaval et al. 1994), the pathogenesis of *Toxocara* has different aspects. Besides the strong polyvisceral form noted in children (Beaver et al. 1952; Glickman et al. 1986), two additional expressions are frequently observed. These involve adults with simple serologic signs but without clinical symptoms, such as those with *Toxocara* infestation or actual toxocariasis.

Toxocara ELISA: diagnostic tool

In view of this clinical complexity and the numerous epidemiologic risk factors involved, the diagnosis of toxocariasis can be made only by serologic methods. The ELISA test, routinely used, is an excellent tool. Although its sensitivity is modest in comparison with that of the Western blot procedure elaborated by Magnaval et al. (1991), we found 100% specificity in our study. Using the same material (ELISA Biokema, Switzerland), Jacquier et al. (1991) have reported 91% sensitivity among a population suspected of having toxocariasis and 14% cross-reactivity among certain types of helminthiasis, including trichinosis, fascioliasis, and strongyloidiasis. Neither our regional study nor our hospital experience confirms these data. Likewise, Nicholas et al. (1986) did not observe any cross-reaction among helminths in an Australian population using the *Toxocara* ELISA test. For routine biologic screening on a European level, the ELISA test retains its value. Western-blotting remains a tool for specialists to use in confirming the diagnosis.

Toxocariasis poses a human health problem in temperate climates. Biologically, it is seen in approximately 5% of the adult European population. Although the expression of *Toxocara* pathogenesis is extremely variable, the disease remains serious due to its ocular involvement. Specific therapy must be introduced in accordance with the epidemiologic-serologic-clinical condition. The therapeutic efficacy can be monitored by the fall in the eosinophil count (Nicholas et al. 1986). *Toxocara* antibodies may persist at high titers for more than one year after effective treatment. Individual and collective prevention measures must be developed through information concerning nutritional habits, hand-washing, and, above all, careful treatment of animal vectors. Although the results obtained in the feline population remain uncertain – primarily due to the widespread abundance of stray cats – the canine population, amounting to more than 10 mil-

lion in France, must be more rigorously surveyed and treated. We advise that a systematic toxocariasis screening be carried out in the native population with lightly symptomatic eosinophilia. This would diminish invasive and costly paraclinical intervention. For the future it would be advisable to establish therapeutic protocols.

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