J. Hyg., Camb. (1983), **90**, 61–65
Printed in Great Britain

Seroepidemiological study on toxocaral infection in man by enzyme-linked immunosorbent assay

By K. MATSUMURA and R. ENDO

Yamaguchi Prefectural Research Institute of Hygiene,
2–5–67 Aoi Yamaguchi 753, Japan

(*Received* 15 *February* 1982; *accepted* 15 *July* 1982)

SUMMARY

A seroepidemiological survey for toxocaral infection was performed using samples from children and adult women in the Yamaguchi area of Western Japan, An enzyme-linked immunosorbent assay using excretory–secretory antigen was applied to these sera. Of samples tested, 3·1 % from children and 3·7 % from women were positive. It was found that regression analysis of positive rates by age between 20 and 70 or more years was significant in the positive direction. The positive rates from urban, rural and fishing areas were 5·7, 3·9 and 1·7 % respectively. Also, the rates from northern, western and eastern parts in the research area were 5·7, 4·7 and 0·5 % respectively. These findings suggested that environmental factors are important for toxocaral infection. Further, the rate for 108 samples who answered that they have owned dogs was 6·2 % compared to 2·9 % of 422 respondents who denied an experience of owning dogs. This fact suggested that attention should be paid to dog breeding for prevention and control of toxocaral infection in man.

INTRODUCTION

Toxocara canis (*T. canis*) infection in man follows ingestion of *T. canis* eggs. The persistence or the migration of *T. canis* larvae in the internal organs of man causes visceral larva migrans (VLM) (Beaver, 1969). Although several nematodes have been associated with VLM, *T. canis* has been most commonly related to this disease. The patient with VLM is usually an infant between 18 months and 5 years of age.

Seroepidemiological studies on human toxocariasis have been carried out by Woodruff and his colleagues (Woodruff, 1970; Woodruff, de Savigny & Jacobs, 1978; de Savigny, Voller & Woodruff, 1979). They reported that of 922 healthy adults tested, 2·6 % were found to have elevated specific antibody levels to toxocaral infection. They also described that the enzyme-linked immunosorbent assay (ELISA) using excretory–secretory antigen (de Savigny, 1975) was useful for detecting the antibodies to toxocaral infection.

This paper concerns a seroepidemiological survey of healthy subjects in Japan. Samples were tested for serum antibody to *T. canis* by using the ELISA procedure. The prevalence of antibodies was determined and the data were analysed for factors related to these samples that might influence the toxocaral infection rate.

MATERIALS AND METHODS

Collection of serum samples and preparation of antigen

The Yamaguchi area, which is located in the western part of Japan, was selected as the research study area. Serum samples from 83 children aged < 1–15 years and from 530 healthy women aged 20–87 years were collected. The sera of children were supplied by Dr T. Suho, paediatrician, and the women's sera were obtained from 15 communities in Yamaguchi Prefecture during November, 1980. At the same time, a questionnaire was given to the women and the following information was requested; name, age, address, living environment and contact with dogs. In particular, we asked the women whether they had owned dogs during the past 10 years.

The excretory–secretory (ES) products of *T. canis* larvae were used as an antigen (de Savigny, 1975). The culture medium of larvae was dialyzed against phosphate buffered saline (PBS) for 48 h and was stored at −20 °C until use.

The enzyme-linked immunosorbent assay

The ELISA was carried out as follows (Matsumura & Endo, 1982). ES antigen was diluted in 0·06 M carbonate buffer, pH 9·6, and the microtitre U plates (Cook no. 223–24) were sensitized overnight at 4 °C by addition of 100 μl of antigen. They were then washed three times with 0·15 M NaCl containing 0·05 % Tween 20. The 100 μl of sera diluted with PBS were added to the wells and the plates were then kept overnight at 4 °C. The sera were removed and the plates were washed as above. Next, 100 μl of conjugate, goat antiserum prepared to human IgG and conjugated to horseradish peroxidase (Miles Laboratories, Inc., Elkhart, USA) was added and incubated for 1 h at 37 °C after being sealed. The plate was washed again as above. 100 μl of substrate, 5-amino salicylic acid (Aldrich Chemical Company Inc., Milwaukee, USA), was then added and incubated for 1 h at room temperature. After stopping the enzyme reaction with 1 N NaOH, the plates were read in a spectrophotometer (Dynatech Laboratories Inc., Alexandria, USA) at 450 nm. The optical density was expressed as an ELISA value.

To determine the end-point titre of this procedure, the positive sera from the patients with visceral and ocular larva migrans and the negative sera collected in our laboratory had been previously tested using ES antigen. For this, the ES antigen was diluted and used at the concentration of 28 μg/ml and the sera were used at a dilution of 1:160 in PBS. The results previously obtained were that the positive and negative sera had the ELISA values of > 0·20 and < 0·06 respectively, i.e. the values of positives were approximately three times the negative. Based on this finding, a significant value in this study was taken to be 0·20 or more.

Age-adjusted rate

In order to eliminate irregularities caused by the different age distribution of sample populations in different areas, results were adjusted to conform to the age distribution of Yamaguchi Prefecture in 1975 (Census of Japan performed in 1975).

RESULTS

Of the 83 samples from children tested, 3 (3·6 %) had antibodies to *T. canis*; one of these was only 9 months of age.

Table 1 shows the positive rates of women by age. Of the 530 samples tested, 3·7 % had antibodies to *T. canis*. Peak prevalence of positives was found to be in the 70 years or older group. No significant difference was observed in the positive rates between children and women. Also, regression analysis of positive rate by age between 20 and 70 or more years showed a statistically significant positive correlation of proportion positive and age ($P < 0·05$).

The results of tests performed on the samples collected from urban, rural and fishing areas in the Yamaguchi area are shown in Table 2. The highest percentage of positive results was found in the samples from urban areas at 5·7 % of the 88 samples. Of samples tested, the positive rates from rural and fishing areas were 3·9 % and 1·9 % respectively, a difference which is not significant. Next, the research area was divided into the northern, western and eastern parts based on the site of 15 communities selected. The results are shown in Table 3. Of the 207 samples from the northern part, 5·7 % were positive, while 4·7 % and 0·5 % were positive from the western and eastern parts, respectively. These results differed significantly ($P < 0·05$).

Table 1. *Age distribution of positive rates and ELISA values to* Toxocara canis *infection in women*

Age (year)	Number tested	% positive	ELISA value*		
			< 0·15	0·15–0·19	≧ 0·20
20–29	69	1·4	61	7	1
30–39	105	2·9	93	9	3
40–49	116	5·2	102	8	6
50–59	117	3·4	107	6	4
60–69	64	4·7	56	5	3
70+	59	6·8	52	3	4
Total	530	4·0 (3·7)†	471	38	21

* ELISA values were given as absorbance at 450 nm and values of ≧ 0·20 were considered to be positive.

† Age-adjusted positive rate was calculated from the age distribution of Yamaguchi Prefecture in 1975.

Table 2. *Positive rates of* Toxocara canis *infection by urban, rural and fishing areas in Yamaguchi, Japan*

Area	Number tested	% positive*
Urban area	88	5·7
Rural area	341	3·9
Fishing area	101	1·9

* Age-adjusted positive rate.

Table 3. *Positive rates of* Toxocara canis *infection by northern, western and eastern parts in Yamaguchi, Japan*

Place	Number tested	% positive*
Northern part	207	5·7
Western part	161	4·7
Eastern part	162	0·5

* Age-adjusted positive rate.

Table 4. *Positive rates of* Toxocara canis *infection for samples with or without an experience of owning dogs*

Ownership*	Number tested	% positive†
Owner	108	6·2
Non-owner	422	2·9

* Ownership was elicited by a questionnaire, i.e. we asked the subjects whether they had owned dogs during the past 10 years.

† Age-adjusted positive rate.

The positive rates for samples from subjects who had at some time owned dogs, and from those who had never owned a dog are shown in Table 4. The rate for 108 subjects who answered that they had owned dogs was 6·2 % compared to 2·9 % of 422 respondents who denied ever owning dogs.

DISCUSSION

Mok (1968) reported that the typical VLM case is in a child aged 1–4 years. In Japan, several cases of VLM in children were reported by Oshima *et al.* (1965) and Yoshimura (1973). However, sero-positive cases in adult women were found in this survey. It is not known from this result whether the elevated ELISA value in these cases is due to adult infection or infection in childhood. However, the regression analysis of positive rates by age between 20 and 70 or more years was statistically significant in the positive direction. This fact suggests that toxocaral infection occurs in adults. Judging from this finding using healthy subjects, it seems that most cases infected with *T. canis* are asymptomatic. However, de Savigny *et al.* (1979) reported that no relation was found between age and ELISA value. In that survey 2·6 % of a healthy population were positive. Also Woodruff (1970) reported that 2·1 % of samples from healthy subjects were positive. These rates were somewhat lower than the rate obtained in this survey.

This survey reveals geographical differences in the positive rates to toxocaral infection. One possible explanation for these differences is that environmental factors such as the climatic or soil conditions are related to the incidence of the infection (Borg & Woodruff, 1973: Quinn, Smith & Bruce, 1980). The highest positive rate in this survey was observed in the urban areas. This fact suggests that the living environment may play an important role in infection with *T. canis*.

Woodruff, De Savigny & Jacobs (1978) reported that 15·7 % of dog breeders and employees at breeding kennels had the high ELISA value. It was also found in this survey that the positive rate in the samples with an experience of owning dogs was greater than in the samples without the experience. These facts suggest that dog handling is an influential factor in toxocaral infection.

We gratefully acknowledge the helpful advice of Dr K. Tanaka and Dr H. Yamagata. Also we are grateful to Dr T. Suho for supplying sera from children.

REFERENCES

BEAVER, P. C. (1969). The nature of visceral larva migrans. *Journal of Parasitology* **55**, 3.

BORG, O. A. & WOODRUFF, A. W. (1973). Prevalence of infective ova of toxocara species in public places. *British Medical Journal* **24**, 470.

DE SAVIGNY, D. H. (1975). *In vitro* maintenance of *Toxocara canis* larvae and a simple method for the production of Toxocaral ES antigen for use in serodiagnostic tests for visceral larva migrans. *Journal of Parasitology* **61**, 781.

DE SAVIGNY, D. H., VOLLER, A. & WOODRUFF, A. W. (1979). Toxocariasis: serological diagnosis by enzyme immunoassay. *Journal of Clinical Pathology* **32**, 284.

MATSUMURA, M. & ENDO, R. (1982). Enzyme-linked immunosorbent assay for Toxocariasis in man. *Bulletin of the Faculty of Agriculture Yamaguchi University*. (In the Press.)

MOK, C. H. (1968). Visceral larva migrans – a discussion based on a review of the literature. *Clinical Pediatrics* **7**, 565.

OSHIMA, T., SATO, K., TANAE, A. & MURATA, M. (1965). The first two cases report of infant Toxocariasis in Japan. *Japanese Journal of Parasitology* **14**, 351.

QUINN, R., SMITH, H. V. & BRUCE, R. G. (1980). Studies on the incidence of *Toxocara* and *Toxocara canis* spp. ova in the environment. 1. A comparison of flotation procedures for recovering *Toxocara* spp. ova from soil. *Journal of Hygiene* **84**, 83.

WOODRUFF, A. W. (1970). Toxocariasis. *British Medical Journal* **3**, 663.

WOODRUFF, A. W., DE SAVIGNY, D. & JACOBS, D. E. (1978). Study of Toxocaral infection on dog breeders. *British Medical Journal* **4**, 1747.

YOSHIMURA, H. (1973). Parasitic disease from pets (dog and cat). *Journal of Medical Technology* **17**, 166.