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Studies of Immunoserological Tests for Acute Human Trichinosis

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Abstract

Serum samples from 474 cases of suspected human trichinosis were examined with complement fixation test (CFT), double diffusion test in agar (DDT) and enzyme-linked immunosorbent assay (ELISA). Among them 77 cases were positive for either of these tests with crude extract of *Trichinella spiralis* larvae. ELISA was the most sensitive method, demonstrating 74 positives out of 77 cases, whereas positives were 35 with CFT and 19 with DDT. A partially purified antigen derived from sticocytes of muscle larvae (Ts-S3) was used on these positive sera for DDT and ELISA. Higher titers were observed in ELISA with Ts-S3 compared with crude antigen. Cross-reactions were observed for other confirmed helminthiases in both of DDT and ELISA with crude antigen, while no cross-reaction was formed with Ts-S3. Furthermore, ELISA with Ts-S3 remained positive for a longer period of time than that with crude antigen.

Key words: *Trichinella spiralis, Selenarctos thibetanus,* immunoserological diagnosis, Ts-S3 fraction

Introduction

World widely distributed trichinosis is one of the most important parasitic zoonoses. In Japan, this disease has been neglected, since the obvious outbreak has not been observed in the past. Recently, however, two outbreaks of trichinosis were reported in Japan. One was that among hunters and their families who had eaten poorly cooked meat of the bear, *Selenarctos thibetanus japonicus,* in Aomori Prefecture in July, 1974 (Yamaguchi, 1975). The other was among presons who were served uncooked meat of the bear, *Urusus arctos yesoensis,* at a restaurant in Hokkaido, in December, 1978 (Obayashi and Yamaguchi, 1981; Ozawa *et al.,* 1981). Thereafter, trichinosis was accounted as a disease which could not be overlooked, even in this country. More recently, the third outbreak was reported in Mie Prefecture (Yamaguchi, 1983). Approximately 500 persons ate uncooked meat of the bear, *S. thibetanus,* imported from China at a restaurant.

Various serodiagnostic tests for trichinosis have been hitherto reported. Kagan and Hillyer (1981) summarized the methods and kit for serodiagnosis of trichinosis available from commercial sources. Enzyme-linked immunosorbent assay (ELISA) was also introduced into the diagnosis for human trichinosis (Au *et al.,* 1983). However, none of these tests was satisfied for the purpose of confirming this disease, because of their high rates of false positive reactions. Since the false positive reactions might be caused by crude antigen, efforts have been made over the past several years to isolate the specific antigen which obviates such reactions. Despommier and Lacetti (1981) showed the advantage of utilizing partially purified antigen prepared from sticocyte secretory granules of muscle larvae. Seawright *et al.* (1983) also reported the out-performance of this antigen in sensitivity, specificity and early detection for swine trichinosis.

The present paper describes the results of

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complement fixation test (CFT), double diffusion test in agar gel (DDT) and ELISA with crude antigen on human cases involved in the latest outbreak of trichinosis in Mie Prefecture. In addition, this paper deals with the comparison between crude antigen and partially purified antigen (Ts-S3) prepared from sticocyte secretory granules of muscle larvae of *T. spiralis* in both ELISA and DDT.

Materials and Methods

Subjects

Serum samples were collected from 474 individuals who had eaten uncooked bear meat imported from China at a restaurant in Mie Prefecture in January, 1982. Follow up examinations were carried out at various intervals on 60 cases with suspected trichinosis up to 46 weeks after infection.

Additional serum samples were obtained from patients with other kinds of helminthiases and from healthy persons as control. Ascariasis, trichuriasis and paragonimiasis westermani were diagnosed by stool examinations previously, and dirofilariasis, angiostrongylosis, gnathostomiasis, paragonimiasis miyazakii and fascioliasis were diagnosed by several immunoserological techniques. The normal sera were collected from 120 students of Chiba University who were proved to be parasite free by stool examinations and had no history of taking raw bear meat.

Parasite and antigens

T. spiralis larvae used for this experiment were of a strain originally derived from the N.I.H., USA., and had been maintained by passage through Wistar rat in our laboratory for 15 years. Infective muscle larvae were obtained by pepsin/HCl digestion (37°C, 2 hrs incubation) of minced mouse carcasses. After washing with distilled water, the larvae were lyophilized and kept at −20°C until used. Crude antigens were prepared from extracts of lyophilized larvae with veronal buffered saline, 0.1% saline, or carbonate buffer (0.1 M, pH 9.6). Ts-S3 antigen was prepared by the method of Despommier and Lacetti (1981), followed by dialysis against carbonate buffer (0.1 M, pH 9.6).

Protein determination

Protein was measured by the Coomassie dye-binding method described by Bradford (1976), using bovine serum albumin as a standard.

Complement fixation test (CFT)

An antibody titer greater than 1:10 dilution of serum samples at a 50% hemolysis endpoint was considered to be a positive reaction (Mayer *et al.*, 1948; Yokogawa and Tsuji, 1962). Veronal buffered saline extracted (VBS) antigen was used at a concentration of 150 μg protein/ml. Reaction mixture (3 ml) contained 0.4 ml each of complement, diluted serum, antigen and hemolysin labeled sheep red blood cells suspension.

Double diffusion test in agar gel (DDT)

For the DDT, 0.1% saline extract was used as antigen (8 mg protein/ml) and 0.9% agarose in pH 8.2 of veronal buffer was employed for the plates. The size of the wells for both antigen and sera were 2 mm in diameter and the distance between the wells was 3 mm (Ouchterlony, 1949).

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed as described by Voller (1976) and Tanaka *et al.* (1979). Carbonate buffer extract was used as antigen, except where otherwise indicated. Each well of plates (Nunc Immuno-plate I) was coated with 100 μl of Antigen (10 μg protein/ml). Peroxidase labelled rabbit anti-human IgG/IgM antibody (Miles-Yeda Ltd.) was used as conjugates. The enzyme substrate consisted of 5-amino salicylic acid in 0.072% distiled waster with 0.005% H_2O_2.

Results

Immunoserological test with crude antigens

Among 474 individuals, 77 were positive for CFT, ELISA or DDT, by single or combination. A total of 35 was positive for CFT. Among them, 33 became positive 30–50 days after ingesting uncooked bear meat, although the

other two who showed typical clinical signs of acute trichinosis were negative on day 33 but became positive on day 73. These 2 cases were negative for DDT during the course of infection but were positive for ELISA at a titer of 1:40 in both examinations. The CFT titers decreased during the course of the infection in most of the cases. The CFT turned to negative in 17 cases 3–4 months after infection including 3 cases which showed the titer of over 1:160 at the first examination. However, 2 cases were still positive 7 months postinfection although they showed titers lower than 1:20 (Fig. 1).

Out of 77 positives for one or more of three examinations, 74 cases were postive for ELISA using anti-human IgG (Table 1), while there was no case being positive with anti-human IgM. The highest titer of ELISA was observed 30–50 days after infection in many cases, although 13 cases showed the highest titer at the 70th day postinfection.

In DDT, 19 were positive with *T. spiraris* larvae antigen. A few DDT positives showed cross-reaction against both of *Toxocara canis* and *Dirofilaria immitis* antigens. These cross-reactions disappeared after absorption with respective antigens. As shown in Table 1 and Fig. 2, out of 74 ELISA positive for CFT and/or DDT; 13 positive for both CFT and DDT, 20 for CFT only and 5 for DDT only. On the other hand, out of 400 ELISA negatives 2 were postiive for CFT and 1 was positive for DDT (Table 1). The number of positives for CFT, DDT or ELISA decreased during the course of the examinations, and in consequence only 2 remained still postive for all of CFT, DDT and ELISA at the 2nd examination carried out on 40–70 days after infection (Fig. 3).

Protein fraction extracted from stichosome of T. spiralis larvae (Ts-S3)

Comparative studies on sensitivity and specificity by using crude and partially purified antigens were carried out on ELISA. Out of 50 cases which showed titers of over 1:40 at the first examination (33–52 days after ingestion

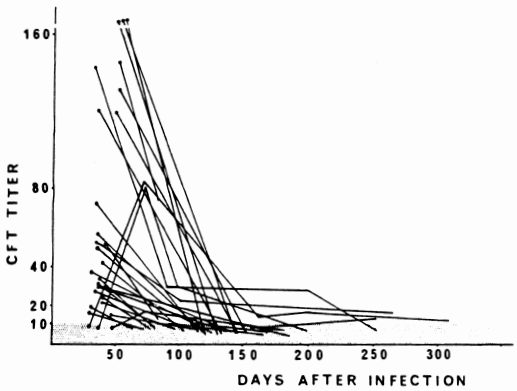

Fig. 1 Changes of antibody titers in CFT.

Table 1 Comparison of results of ELISA, CFT and DDT with crude antigens from *Trichinella spiralis* muscle larvae

	ELISA +	ELISA −	Total
CFT+ DDT+	13	0	13
CFT+ DDT−	20	2	22
CFT− DDT+	5	1	6
CFT− DDT−	36	397	433
Total	74	400	474

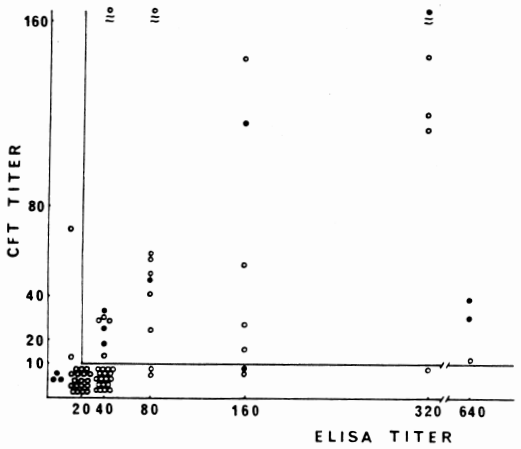

Fig. 2 Comparison of the results with CFT, DDT and ELISA at the first serum examination.
• DDT positive, ○ DDT negative

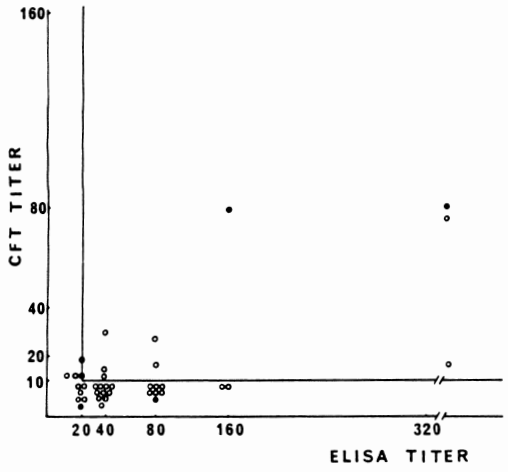

Fig. 3 Comparison of the results with CFT, DDT and ELISA at the second serum examination.
• DDT positive, ○ DDT negative

of bear meat) with the curde antigen, 35 cases showed the titer of over 1:40 with Ts-S3. At the third examintion (98–188 days after ingestion), 18 with the crude antigen and 17 with Ts-S3 shwoed the titer of over 1:40. Of these samples, 7 with crude antigen and 14 with Ts-S3 showed over 1:80 in the titer. There was a significant difference between the results with the crude antigen and Ts-S3 in the number of cases with high titers ($p \leqq 0.05$, Table 2).

Moreover, corss-reactivities of the cude and partially purified antigens were examined for the ELISA and DDT using serum samples of patients with various helminthiases. A serum sample with a CFT titer of 1:450 which was obtained from a patient who was diganosed as

Table 2 Comparison of *T. spiralis* crude antigen and Ts-S3 antigen in ELISA

	1st Exam.*		3rd Exam.†	
	crude	S3	crude	S3
1: 40	26	9	11	3
1: 80	9	8	5	10
1: 160	7	8	2	3
>1: 320	8	10	0	1
Total	50	35	18	17

*Examined at 33–52 days after infection
†Examined at 98–188 days after infection

trichinosis by muscle biopsy (Ozawa *et al.*, 1981), and 2 serum samples with the titer of over 1:80 in CFT obtained in the present study were used as reference serum for trichinosis.

The results of ELISA on these reference samples were positive with the titer of over 1:80 against both crude antigen and Ts-S3. Strong precipitin bands appeared between the serum and both antigens in DDT. In ELISA, all sera from patients with ascariasis, angiostrongylosis or paragonimiasis miyazakii were negative with the crude antigen. However, the sera from individuals with trichuriasis, mixed infections with ascariasis and trichuriasis, paragonimiasis westermani, dirofilariasis, gnathostomiasis or fascioliasis showed titers ranging from 1:40 to 1:320 with the crude antigen in ELISA (Fig. 4). As shown in Fig. 4, cross-reactions were also observed in DDT when the crude antigen was used against several sera of patients with dirofilariasis, gnathostomiasis, paragonimiasis westermani or fascioliasis. Nevertheless, these cross-reactive sera were all negative in both ELISA

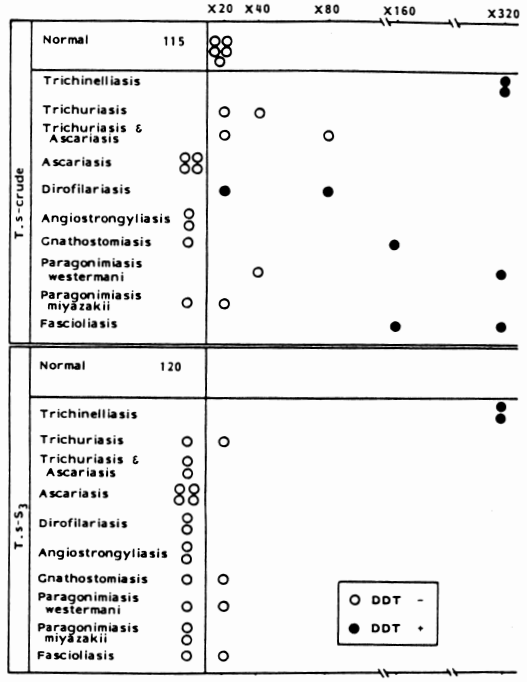

Fig. 4 Results of ELISA and DDT with T.s crude antigen and T.s-S3 antigen against serum samples from patients with proven helminthiases.

and DDT when Ts-S3 was used. Of 120 normal serum samples, 5 showed a false positive reaction with a titer of 1:20 in antibody titer in ELISA with crude antigen (Fig. 4).

Discussion

The only practical means of diagnosis for trichinosis is to demonstrate antibodies to *T. spiralis*, when the direct detection of larvae is difficult by muscle biopsy. Pleural muscle probes were carried out on 11 individuals of trichinosis in Aomori and Hokkaido with negative results, although a muscle larva was detected from only one patient as yet (Ozawa *et al.*, 1981). There are numerous reports in the literatures concerning the immunoserological diagnosis on human trichinosis (Lawniczak *et al.*, 1979; Au *et al.*, 1982; Yamaguchi *et al.*, 1979; van Knapen *et al.*, 1982). However, the definitive and reliable immunoserological technique has not yet been developed.

In the present study three kinds of immunoserological tests, *i.e.*, CFT, DDT and ELISA, were performed on 474 suspected cases with *T. spiralis* infection by using crude antigen. Out of 77 positives, 41 were positive for a pair of these tests, while 13 were positive for all the tests (Table 1). However, the results of CFT, DDT and ELISA did not always correspond each other.

As far as we have examined, usually in almost all DDT positive cases of helminthiases, such as paragonimiasis, schistosomiasis, fascioliasis, angiostrongylosis, dirofilariasis, toxocariasis and gnathostomiasis, were also positive for both CFT and ELISA. However, ELISA positives were not always positive for CFT and DDT. Accordingly, there is no doubt about a possibility that there were some false positives in ELISA.

On the other hand, it was revealed that 2 CFT positives were negative for ELISA, and 5 DDT positives were negative for CFT in this study (Table 1). It is very interesting that the titer of these examinations with the crude antigen decreased immediately in every cases with trichinosis (Figs. 1, 3). On the contrary, in the case of paragonimiasis, it is known that CFT remains positive with a high antibody titer for a few years if any chemotherapy is not performed (Yokogawa *et al.*, 1961).

Comaprison of the sensitivity and specificity between *T. spiralis* crude antigen and partially purified Ts-S3 antigen was carried on both DDT and ELISA by using serum samples from patients with another kind of helminthiases. ELISA with Ts-S3 on reference serums showed a stong positive reaction with titers of over 1:320 (Fig. 4). There was a tendency that ELISA titers with Ts-S3 persisted for a longer period of time than those with crude antigen did (Table 2). Furthermore, no cross-reaction was observed on other helminthiases when Ts-S3 was used as coating antigen. These facts suggest that utilization of Ts-S3 may be a major improvement in the immunoserological diagnosis for human trichinosis.

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