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Detection of Human and Rabbit Antibodies to *Trichinella spiralis* by Enzyme Linked Immunosorbent Assay (ELISA)

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Abstract

ELISA method was applied to detection of antibodies from infected rabbit sera and patient sera by using antigens of *T. spiralis* strains isolated in Japan, Thailand and Poland. Iwasaki strain (Japan) infected rabbit serum showed higher ELISA values than normal serum, and were highly reactive with the homologous antigen. Patient serum at the onset of trichinellosis in Thailand had significantly higher ELISA values than these of the normal human serum. The serum was most reactive with Thai antigen and secondly with Iwasaki antigen. At 265 days PI, patient serum had still high titers. The titer only against Thai antigen decreased to the same level of Iwasaki antigen.

Key words: *Trichinella spiralis*, ELISA, Trichinellosis, zoonosis

Introduction

Trichinellosis caused by *Trichinella spiralis* is one of the most important zoonoparasitic diseases. Generally it is caused by ingesting inadequately processed pork or meat of bears, and occurs world wide (Steele, 1981). Although in Japan only three outbreaks have been reported by eating domestic or imported bear meat (Yamaguchi, 1982), there will be many possibility of infection of Japanese travelers abroad or outbreaks by imported meat. It is necessary to establish early diagnosis methods.

The most satisfactory diagnosis of trichinellosis is to detect muscle larvae directly from patient muscle by biopsy. In order to improve the poor sensitivity of the direct method, many immunological methods have been attempted (Despommier, 1986). Recently, by the advantage of rapidity and amenability to automation, ELISA methods for detecting specific antibodies of human or swine sera have been intensively investigated in U.S. and European countries.

In the present study, it was tried to detect antibodies from infected rabbit sera and patient sera by the ELISA method by using antigens of *T. spiralis* strains isolated in Japan, Thailand and Poland.

Materials and Methods

Strains of T. spiralis: Three strains of *T. spiralis*, Japanese strain (Iwasaki strain; I-strain) originated from Hirosaki University in Japan, Polish strain (P-strain) from the Ruks Institute in the Netherlands and Thai strain (T-strain) from the University of Chiang Mai in Thailand, were used. Details of these strains were mentioned previously (Ogimoto, 1984a).

Preparation of soluble antigens: Soluble antigens were prepared by the method as described previously (Ogimoto, 1984a). It is briefly mentioned as follows;

Infective larvae freshly isolated from infected mice were lyophilized, delipidized and ground in a teflon homogenizer. The homogenate was centrifuged, and sediment was emulsified with vernal bicarbonate buffered saline.

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After extraction at 4°C for 3 days, supernatant was collected as a soluble antigen.

Human sera and rabbit sera: Patient sera were obtained from a man who was infected with *T. spiralis* in the northern area of Thailand and normal serum was obtained from a man who had no record of any parasitic diseases in Japan. Infected rabbit serum was obtained from a rabbit infected orally with 10,000 larvae of *T. spiralis* I-strain.

ELISA: ELISA was performed by modified methods of Voller *et al.* (1976). Each well of 96 well-microtiter plastic plates (Sumitomo Bakelite Co.) was coated with 100 μl soluble antigens in 0.05 M carbonate buffer (pH 9.6) at 4°C overnight. After antigen solution was removed, wells were coated with 200 μl 0.1% bovine serum albumin in phosphate-buffered saline (PBS) at 4°C overnight. Wells were then washed 5 times with PBS containing 0.1% Tween 20 (PBST). Sera diluted in PBST were added to the wells (100 μl/well). After 1.5 h of incubation at room temperature, the wells were washed 4 times with PBST and then incubated with alkaline-phosphatase-conjugated goat IgG anti-rabbit IgG or peroxidase-conjugated goat IgG anti-human IgG+IgA+IgM+IgD (Miles-yeda LTD.) in PBST (100 μl/well). After washing 5 times with PBST, the reaction was developed with p-nitrophenyl phosphate or phenylendiamine for 30 min at room temperature. Absorbances were measured by a Microelisa Reader (Dynatech Laboratories).

Results

Reactivity of a normal rabbit serum to *T. spiralis* I-strain antigen (I-antigen) was investigated by ELISA (Table 1). Wells with antigen under 1 μg/ml showed negligible level of the values, although wells coated with 5 μg/ml or more antigen showed relatively higher values. Similar results were observed in T-strain antigen (T-antigen) and P-strain antigen (P-antigen).

Table 2 showed ELISA values of I-strain-infected rabbit serum. The serum indicated higher values against each of three antigens than the normal rabbit serum. ELISA values against I-antigen were higher than values against T- or P-antigens at almost every combination of antigen concentration and serum dilution. P-antigen showed the lowest values among these three antigens.

Sera obtained from a normal human and a patient in Thailand at the onset of trichinellosis and at 265 days PI were investigated by the ELISA (Table 3). ELISA values of the normal serum at 500 times dilution were negligible level against any of three antigens, while ELISA values of the serum at 50 times dilution was relatively high.

Patient serum showed high values at the onset of infection. The values of the patient serum at 5,000 times dilution were higher than those of the normal serum at 50 times dilution. This suggests that the patient has more than 100 times higher titer than that of the normal human.

At 265 days PI, values at 5,000 times dilu-

Table 1 ELISA values of a normal rabbit serum sample against *T. spiralis* I-antigen

Antigen concentration (μg/ml)	serum dilution (X)			
	20	40	80	160
0.1	0.006	0.003	0.004	0.004
0.5	0.032	0.012	0.007	0.006
1.0	0.056	0.028	0.017	0.011
5.0	0.107	0.087	0.043	0.034
10.0	0.098	0.070	0.052	0.040
50.0	0.116	0.091	0.057	0.042

Table 2 ELISA values of *T. spiralis*-I-strain-infected rabbit sera against *T. spiralis* antigens

Antigens (μg/ml)	serum dilution (X)			
	20	40	80	160
I-antigen				
0.1	0.215	0.218	0.206	0.132
0.5	0.661	0.742	0.672	1.162
1.0	1.157	1.078	1.088	1.172
5.0	over	over	over	over
10.0	over	over	over	over
50.0	over	over	over	over
T-antigen				
0.1	0.120	0.184	0.134	0.067
0.5	0.857	0.933	0.819	0.659
1.0	0.787	0.841	0.691	0.958
5.0	over	over	1.414	over
10.0	over	over	1.189	over
50.0	over	over	over	over
P-antigen				
0.1	0.066	0.026	0.044	0.091
0.5	0.417	0.382	0.357	0.443
1.0	0.796	0.596	0.659	0.767
5.0	1.145	1.025	1.095	1.263
10.0	1.056	0.899	0.982	1.272
50.0	1.046	1.005	0.755	1.186

tion had decreased to the negligible level. Values of the serum at 500 times dilution, however, were higher than the values of 50 times diluted normal human serum at almost of antigen concentrations.

Titers of the patient sera were compared by using the same concentration of three antigens (1 μg of protein / ml) (Table 4). When 0.100 was regarded as the endpoint, the titer of the patient serum at the onset of infection was 32,000 times against T-antigen, 16,000 against I-antigen and 8,000 against P-antigen. At 265 days PI the titer against T-antigen decreased to 16,000 times. Titers against other antigens, however, had not decreased by 265 days PI.

Discussion

ELISA methods have been applied to the detection of *T. spiralis*-specific antibodies from human sera or swine sera. Many authors used supernatant of larvae homogenate as the antigen for ELISA (Ruitenberg *et al.*, 1974; Engvall and Ljungström, 1975; Ruitenberg *et al.*, 1976; Ruitenberg and Van Knapen, 1977; Saunders *et al.*, 1977; Clinard, 1979; Van Knapen *et al.*, 1980; Van Knapen *et al.*, 1981; Au *et al.*, 1983; Faubert *et al.*, 1985). In these systems using crude antigens, problems of the false positive have not been overcome (Van Knapen *et al.*, 1984). ELISA using antigens purified by Sephadex G-200 (Taylor *et al.*, 1980), immunoaffinity chromatography liganded with hy-

Table 3 ELISA values of normal and patient sera against *T. spiralis* T-antigen

serum	Antigen concentration (μg/ml)	serum dilution (X)		
		50	500	5,000
Normal	0.1	0.028	0	0
human	0.5	0.128	0.004	0
	1.0	0.185	0.012	0
	5.0	0.324	0.026	0
	10.0	0.271	0.026	0
Patient	0.1	0.355	0.335	0.108
onset	0.5	over	1.286	0.307
	1.0	over	1.299	0.385
	5.0	over	over	0.669
	10.0	over	over	0.492
Patient	0.1	0.146	0.003	0
265 days	0.5	0.899	0.227	0
PI	1.0	1.111	0.276	0
	5.0	over	0.517	0.027
	10.0	over	0.537	0.019

Table 4 ELISA values of patient sera at the onset or 265 days after *T. spiralis* infection

Antigens*	collection of sera†	serum dilution (X)							
		2,000	4,000	8,000	16,000	32,000	64,000	128,000	256,000
I-antigen	onset	0.805	0.398	0.246	0.123	0.065	0.033	0.013	0.007
	265 days	0.838	0.427	0.269	0.157	0.079	0.030	0.016	0.018
T-antigen	onset	0.984	0.609	0.394	0.234	0.132	0.072	0.025	0.013
	265 days	0.786	0.418	0.244	0.135	0.073	0.025	0.016	0.001
P-antigen	onset	0.622	0.329	0.192	0.071	0.046	0.016	0.001	0.000
	265 days	0.474	0.326	0.180	0.096	0.054	0.018	0.061	0.028

*: Each antigen was coated at the concentration of 1 μg of protein / ml.
†: Sera were collected from a patient at the onset or 265 days after *T. spiralis* infection.

perimmune rabbit sera (Seawright *et al.*, 1983) or immuno-affinity chromatography with monoclonal antibodies (Gamble and Graham, 1984a, b; Silberstein and Despommier, 1984), showed high sensitivity and low noise of non-specific or cross reactions. However, if purified antigens used were strain-specific, i.e. lacked species-specific epitopes of *T. spiralis*, it is impossible to detect antibodies against other strains with different antigenicity by the ELISA system using such purified antigens.

In the present paper, we used crude antigens, and detected antibodies against *T. spiralis* from the rabbit serum and the human sera (Tables 2, 3, 4). The noise which may come from non-specific reaction or cross reaction of sera could not be neglected at higher concentration of antigens or sera. It will not be easy to reduce

the noise in the ELISA system by using the crude antigens. However, the system detect antibodies of high titer of patient sera easily. Therefore, the ELISA system has a possibility for being used as the convenient method of diagnosis of the trichinellosis.

The rabbit infected with *T. spiralis* I-strain was highly reactive with the homologous antigen (I-antigen), compared with the heterologous antigens (T- and P-antigens) (Table 2). The Thai patient showed the higher titer (32,000) against T-antigen than titers against I-antigen (16,000) or P-antigen (8,000) (Table 4). These facts suggested that there are strain-specific antigenic differences between the three strains of *T. spiralis*. This corresponds well with the previous work of Ogimoto (1984a, b) on antigenic differences among I-, P- and T-strain indicated by the indirect immunofluorescent antibody (IIF) test or epiimmunofluorescent antibody test. This agreement may suggest that the antigens reactive with the antibodies in the ELISA are the same as the antigens detected by IIF.

The titer of the patient serum decreased against T-antigen at 265 days PI, but did not against I- and P-antigens (Table 4). This may suggest that strain-specific antibodies disappear earlier than species-specific antibodies in the course of recovery of the disease.

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