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Research Note

Application of IgG-ELISA for Mass Screening of *Paragonimus* and *Gnathostoma* Infections in the Central Part of Miyazaki Prefecture, Japan

WONG-YOUNG CHOI[1)], JUN-ICHI IMAI[2)], YOICHIRO HORII[2)] AND YUKIFUMI NAWA[2)]

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Miyazaki Prefecture had been known as the endemic area of paragonimiasis westermani and over 300 cases were discovered in 1955–1960 (Hayashi, 1978). Although the prevalence of this disease in Miyazaki Pref. drastically decreased during a period of 1970–1980, the number of sporadic cases are increasing with time for recent 5 years mainly in the central to the southern part (Matsuoka *et al.*, 1986; Ichiki *et al.*, 1989; Ogata *et al.*, 1990a; Nabeshima *et al.*, 1991). Miyazaki Pref. is also known as the endemic area of gnathostomiasis doloresi. Since the first confirmed human case of this disease in the world was recorded by us (Ogata *et al.*, 1988; Nawa *et al.*, 1989), over 10 cases were found in the central part of Miyazaki Pref. (Ogata *et al.*, 1990b). Paragonimiasis and gnathostomiasis are both food-borne parasitic diseases and the life cycles of both pathogens are closely related to wild boars. Since both diseases are endemic in the central part of Miyazaki Pref., we have carried out a preliminary study of sero-epidemiological survey for these diseases in the endemic area.

The sera examined in this study were the stock (155 males, mean age: 54.1 year-old, range: 25–85 y.o.; 192 females, mean age; 53.1 y.o., range 27–87 y.o.) collected from more than 80% of the habitants of Hae, Itaya, Yokono, and Koshinoo of Nishi-Mera Village, Koyu-County, Miyazaki Pref., in 1980 when general health survey was carried out by the Department of Public Health, Miyazaki Medical College. Sera obtained from 100 healthy volunteers of the students of Miyazaki Medical College served as negative control. In addition, pooled sera obtained from the confirmed cases of paragonimiasis westermani and of gnathostomiasis doloresi recently found in Miyazaki prefecture served as positive controls. All serum samples were kept at −30°C until used.

Adult worms of *Paragonimus westermani* (Pw) were obtained from experimentally infected cats. The worms were lyophilized and extracted with saline to obtain an antigen preparation according to the method described previously (Lee and Choi, 1983). *Gnathostoma doloresi* (Gd) adult worms were obtained from the stomach of naturally infected wild boars. The methods for preparing crude antigen was same as those used for Pw.

Enzyme-linked immunosorbent assay (ELISA) was performed according to the method of Matsuda *et al.* (1984) with slight modification. In brief, each well of microtiter plates (Dynatech,

[1)]*Department of Parasitology, Catholic University Medical College, Seoul, Korea.*
[2)]*Department of Parasitology, Miyazaki Medical College, Kiyotake, Miyazaki 889-16, Japan.*
崔　源永（大韓民国カトリック医科大学寄生虫学講座
今井淳一　堀井洋一郎　名和行文（宮崎医科大学寄生虫学講座）

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M129A) was sensitized by incubating either 0.1 ml of Pw antigen (2 μg protein/ml) or Gd antigen (5 μg protein/ml) in 0.05 M bicarbonate buffer (pH 9.6) at 4°C overnight. After 3 successive washings with saline containing 0.1% Tween-20, free binding sites of each well was blocked by a blocking buffer (×4 diluted Block Ace®: Yukijirushi Daily Products Co., Sapporo, Japan). The wells were washed three times and 0.1 ml of serum, diluted 1:100 with the blocking buffer, was added to each well. After incubation at 37°C for 1 h, the wells were washed 3 times and then 0.1 ml of horseradish peroxidase-labelled anti-human IgG rabbit serum (gamma chain specific, Miles-Yeda), diluted 1:2000, was added to each well. The plates were incubated at 37°C for 1 h. After washing enzyme reaction was performed at 25°C for 1 h, using 2,2-azino-di-(3-ethylbenzthiazoline sulfonic acid) (ABTS, Sigma) as the substrate. The absorbance of each well was read by the Titertek Multiscan at the wave length of 405 nm. ELISA values were expressed as optical density (OD) at 405 nm. Cut-off points for positive cases were arbitrarily determined as the mean + 3SD of normal controls. At these cut-off points, all normal control sera were negative against *P. westermani* and only 1 sample was marginally positive against *G. doloresi*.

Results are summarized in Table 1. By IgG-ELISA against two parasite antigens, 8 (2.3%) out of 347 samples were positive for *P. westermani*, while 27 (7.8%) were positive against *G. doloresi*. When the ELISA values of individual sample against two parasite antigens were plotted, only two cases were positive against both parasites (Fig. 1). Among 8 cases of *P. westermani*-positive cases, 4 cases showed relatively high ELISA value (>0.500) and 4 cases were marginal. Two cases of *P. westermani*-positive males who showed high ELISA value (>0.600) were also positive by an Ouchterlony's double diffusion test (data not shown). Although nearly 8% of the samples were positive against *G. doloresi*, their ELISA values were mostly marginal and only one case (male) showed high ELISA value against *G. doloresi*.

These results indicate that more uncovered cases should exist in this area. With certain limitations, ELISA is considered as a sensitive, simple and reproducible diagnostic technique for parasitic diseases (Voller *et al.*, 1976). Concerning paragonimiasis, applicability of ELISA for the diagnosis has been repeatedly reported (Knobloch and Lederer, 1983; Yokogawa *et al.*, 1983). Recently one of us (Imai, 1987) reported that ELISA for paragonimiasis is not only advantageous for the mass screening but also for the evaluation of the efficacy of the treatment.

Table 1 Number and frequency of ELISA-positive cases in Nishi-Mera Village

Group	No.	No. of positives* against	
		P. westermani	*G. doloresi*
Male	155	7 (4.5%)	15 (9.7%)
Female	192	1 (0.5%)	12 (6.3%)
Total	347	8 (2.3%)	27 (7.8%)

*: ≥ mean + 3SD of control

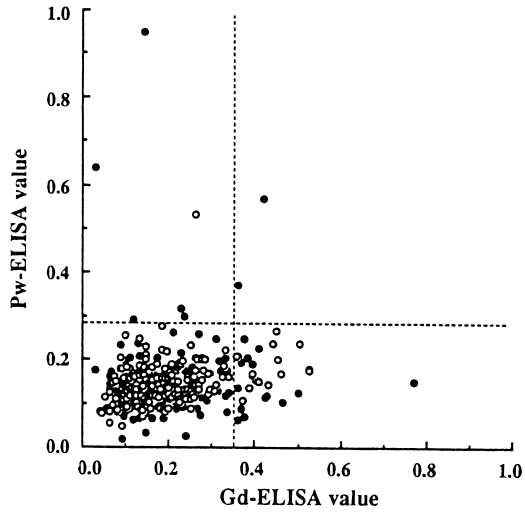

Fig. 1 Correlation of the ELISA values of individual serum sample against *P. westermani* and *G. doloresi* antigens
●: Male, ○: Female
Dotted lines represent cut-off points (Mean + 3SD of the control)

Applicability of ELISA for the diagnosis of gnathostomiasis is also well documented in highly endemic areas such as Thailand (Suntharasamai *et al.*, 1985; Maleewong *et al.*, 1988) and Ecuador (Mimori *et al.*, 1987). In the present study, majority of gnathostomiasis-positive cases had ELISA values of only slightly higher than the arbitrarily determined cut-off point. Whether or not these marginally positive cases have had a history of actual infection should be determined in future. The serum samples were obtained where various helminthiasis are still endemic (Ishii *et al.*, 1980). Since the ELISA using crude *Gnathostoma* antigen showed a certain degree of cross reactivity against sera obtained from various helminthiasis patients (Suntharasamai *et al.*, 1985), some, even if not all, of marginally positive cases in this study may be due to the cross reactivity. So far we could see from the present results, cross reactivity between *P. westermani* and *G. doloresi* antigens seem to be extremely low because double positive cases were rare as shown in Fig. 1. Identification of specific antigens of *G. doloresi* and its application for ELISA is required to establish reliable immunoserodiagnostic method for gnathostomiasis.

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