

DETECTION BY IMMUNOBLOT ASSAY OF ANTIBODIES TO *TAENIA SOLIUM* CYSTICERCI IN SERA FROM RESIDENTS OF RURAL COMMUNITIES AND FROM EPILEPTIC PATIENTS IN BALI, INDONESIA

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Abstract. Several studies from Bali have indicated the presence of *Taenia solium*. Relatively little has been reported, however, implicating human exposure to this parasite on Bali based upon the prevalence of anti-*T. solium* antibodies in asymptomatic and epileptic individuals. This study was conducted to determine by immunoblot assay and ELISA the frequency of anti-cysticercus antibodies in two groups of Balinese. Among 746 residents of four ecologic zones, 94 (13%) were positive by immunoblot. Of 74 epileptic patients from throughout the island, 10 (14%) were positive by immunoblot and 8 (11%) by ELISA; however, only 4 (22%) of the 18 sera positive in either test were positive in both assays. The previously defined high specificity and sensitivity of immunoblot indicates that *T. solium* cysticercosis is well established in Bali and that a significant amount of epilepsy may be due to neurocysticercosis.

INTRODUCTION

Bali is one of the smaller islands in the Indonesian archipelago. About 80% of its population is rural and live in communities that only infrequently have potable water or adequate fecal disposal (Sutisna, 1989; Suweta, 1991). A large proportion of households raise pigs that commonly roam freely. In addition, raw or undercooked pork and beef are consumed regularly in ceremonial and domestic preparations called "lawar". Such factors typically contribute to high levels of *Taenia solium* and *T. saginata* tapeworm infestations, and to *T. solium* induced cysticercosis.

Although several reports have indicated that *T. solium* transmission occurs in Bali (Simanjuntak *et al*, 1977; Coker-Vann *et al*, 1981, 1984; Widjana and Kapti, 1983; Sutisna, 1989; Suweta, 1991) relatively little is known about human exposure to the parasite as reflected by prevalence of anti-*T. solium* antibodies or of neurocysticercosis. In the only published serologic survey, Coker-Vann *et al* (1981) reported that in

the hamlet of Tenganan 21% of 53 sera tested were positive by ELISA. In a 1977 questionnaire, 2% of 2,500 persons reported passing a tapeworm (Widjana and Kapti, 1983). Worm identifications in several reports showed that *T. saginata* was much more common than *T. solium* (Sutisna, 1989). Studies have not been conducted in Bali to determine the presence of the newly recognized Taiwan *Taenia* strain (*T. asiatica*) (Ito, 1992).

The research reported here was to evaluate human infection with *T. solium* cysticerci by determining the frequency of anti-cysticercus antibodies in two groups of Balinese: 746 residents of four ecologic zones and 74 epileptic patients from various communities on the island. For this purpose, immunoblot and ELISA were used, immunologic assays that have been standardized (Espinoza *et al*, 1986; Tsang *et al*, 1989) and also applied in epidemiologic surveys (Coker-Vann *et al*, 1981; Diaz-Camacho *et al*, 1990, 1991; Dumas *et al*, 1989; Sarti *et al*, 1988).

MATERIALS AND METHODS

The population of Bali in 1991 was 2.7 million persons. Blood specimens were collected in 1989

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(Sutisna, personal communication) from residents of four subdistricts, each subdistrict being representative of a separate ecologic zone in Bali, based on elevation and annual rainfall. From the original randomized survey of 2,410 persons, 746 sera were available for testing in this study. An additional 74 sera were obtained from epileptic patients seen at several Bali hospitals and clinics. Sera were separated by centrifugation and kept frozen until tested in the laboratory of A. Flisser. Immunoblot was performed as described by Tsang *et al* (1989), with some modifications. The antigen was a lentil-lectin glycoprotein fraction derived from *T. solium* cysticerci taken from pigs in Mexico and was mixed with 0.1% beta mercaptoethanol, the slab gel was prepared with 10% acrylamide and 0.8% bis-acrylamide, the buffer system for electrophoresis was 0.02 M Tris, 0.15 M glycine with 10% SOS and the conjugate used was goat anti-human IgG coupled to horseradish peroxidase (Zymed Labs). This EITB assay gave similar sensitivity and specificity values as the original assay when sera from patients with neurocysticercosis and sera from pigs with experimental cysticercosis were analyzed. ELISA was conducted as described by Espinoza *et al* (1986), using a crude antigen from similar *T. solium* cysticerci.

RESULTS

In the survey, 94 (12.6%) of the 746 sera tested were positive by immunoblot (Table 1). By age group, there was a gradual, though non-significant increase in seropositive rates through age group 21-30 years. By gender 12% of 334 sera from males and 13% of 412 sera from females were positive, a non-significant difference. The four subdistricts in which the subjects lived were: Kerambitan (low, wet) 200 meters elevation, 2300 mm/year rainfall, 274 sera examined, 35 positive (13%); Batarita (high, wet) 700 m 1,500 mm, 181 sera examined, 25 positive (14%); Kabupaten (low, dry) 300 m, 900 mm, 217 sera examined, 26 positive (12%); Kentamanae (high, dry) 800 m, 900 mm, 74 sera examined, 8 positive (11%). There was no significantly different seropositive rate between any of these subdistricts.

Of the 74 epileptic patients (ages 10-59 years) there were 28 females examined, 3 (10.7%) seropositive and 46 males examined, 7 (15%) seropositive by immunoblot. There was no significant difference between genders (Table 1).

Comparing the results obtained by ELISA with those from immunoblot, overall there were 10 (13.5%)

Table 1

Age and gender-specific *T. solium* cysticerci antibody prevalence by immunoblot assay with sera from asymptomatic and epileptic individuals.

	Asymptomatic individuals			Epileptic individuals		
	No. tested	No. positive	(%) +	No. tested	No. positive	(%) +
Age group						
0-5	106	8	7.5	0	0	0
6-10	207	23	11.1	2	0	0
11-20	262	38	14.5	21	1	4.8
21-30	78	13	16.6	25	7	28.0
31-41	39	4	10.3	22	1	4.5
> 41	54	8	14.8	4	1	25.0
Gender						
Female	412	54	13.6	28	3	10.7
Male	334	40	12.0	46	7	15.2
Total	746	94	12.6	74	10	13.5

positive by immunoblot and 8 (10.8%) by ELISA (Table 2). However, the percent agreement between the two test methods used in this work on an individual sera basis was quite variable. Thus, of the 10 positive sera by immunoblot, only 4 were also positive by ELISA (40% agreement) while of the 64 sera negative by immunoblot, 60 were negative by ELISA (94% agreement) (Table 2).

With regard to glycoprotein (GP) band detection of the 94 sera positive from the asymptomatic population surveyed, 90% of these sera reacted either with antigen GP50 or 42 or both. A small number of samples also reacted with GP 24, 21, 18, 14, or 13 (Table 3). Of the 10 seropositive epileptic patients 8 sera reacted with GP50 or 42, while one serum sample reacted with both bands. Three of the 8 sera also reacted with GP24. Only one serum sample from the epileptic patients reacted only to GP18 (Table 3).

DISCUSSION

In our samples from four ecologic regions on Bali, the overall prevalence for anticysticercus antibodies

Table 2

Comparison of the immunoblot assay and ELISA results obtained by testing sera from the 74 epileptic patients.

Code No.	Immunoblot GP bands	ELISA
1	50, 42, 24	+
2	50, 24	-
3	50	-
4	50	-
5	42, 24	+
6	42	+
7	42	-
8	42	-
9	18	+
10	14	-
11	--	+
12	--	+
13	--	+
14	--	+
15-74	--	-
No. tested	No. positive	No. positive
74	10	8

Table 3

Frequency of the seven specific glycoprotein bands in the immunoblot assay with sera from the two groups of subjects.

Subjects (No. seropositive)	Band	Frequency		Cumulative %
		No.	%	
Asymptomatic individuals (94)	GP50	56	60	93
	GP42	31	33	
	GP24, 18	7	7	
	GP21	6	6	
	GP14, 13	1	1	
Epilepsy (10)	GP50	4	40	90
	GP42	5	50	
	GP24	3	30	
	GP18	1	10	
	GP14	1	10	

was 13%. Since the immunoblot assay has been shown to be 100% specific and 98% sensitive (Tsang *et al*, 1989), our seropositive findings indicate that in the population studied a high proportion are or have been infected with cysticerci of *T. solium*. The nearly uniform distribution of seropositivity in the population studied, by age, gender, and geoclimatic zones suggests that populations with high antibody levels to *T. solium* cysticerci occur elsewhere on the Island. This is likely due to very similar living conditions and behavioral factors Island wide that contribute to *T. solium* transmission. The presence of anti-cysticercus antibody in 14/74 (19%) of the epileptic patients studied serologically also suggests a broadly based infection on the Island.

This report provides the first information on the frequency of seropositivity to *T. solium* and epilepsy in Bali and suggests that a significant amount of epilepsy may be due to neurocysticercosis. Confirmation will require evaluation of epileptic patients by imaging methods. The only other information available on epilepsy in Bali are frequency rates of 1-8%, reported in four communities (Simanjuntak *et al*, 1977; Sutisna, 1989).

Although ELISA provides a simpler and quicker

method than immunoblot (EITB) for examining large numbers of serum samples the former method, when used for *T. solium* seroepidemiology has been shown to have several drawbacks. There are cross reactions with the current ELISA antigens (Espinoza *et al*, 1986; Schantz *et al*, 1980). There is a low positive predictive value with ELISA (Ramos-Kuri *et al*, 1992), and the test has been previously determined unreliable in seroepidemiologic surveys (Diaz-Camacho *et al*, 1991). As shown by the work reported here, there was a lack of agreement between the two methods when the 74 samples from epileptic patients were examined. Our results support the view that ELISA, at the present time is only useful for confirmation of neurocysticercosis in clinical cases (Alarcon-de Noya *et al*, 1989). For these reasons we did not test the 746 sera from asymptomatic individuals by ELISA, preferring to use the EITB method.

This study has also demonstrated that both asymptomatic and epileptic seropositive individuals reacted in at least 90% of the cases to either GP50 or GP42 or both, and only infrequently to other specific GP bands. Such consistency suggests that these two purified GP alone will be sufficient to provide adequate sensitivity for future seroepidemiological studies of cysticercosis in Bali. Previous studies have also shown that in other human populations, GP50 and 42 as well as 24 are most frequently recognized (Tsang *et al*, 1989; Feldman *et al*, 1990). These findings indicate that additional purification of GP50 and GP42 might provide, as a combined antigen, a convenient and highly reliable means of screening large numbers of individuals, via a plate or dot ELISA system, for cysticercosis.

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