

SEROEPIDEMIOLOGY OF FIVE MAJOR ZONOTIC PARASITE INFECTIONS IN INHABITANTS OF SIDOARJO, EAST JAVA, INDONESIA

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Abstract. We conducted a seroepidemiological survey of zoonotic parasite infection in inhabitants of East Java, Indonesia. The subjects of the survey were 244 persons selected from visitors to Sidoarjo City Hospital in East Java between May 1992 and October 1993. Ninety-seven had diarrhea and the rest came to the hospital for routine check-ups. All serum samples were tested for antibodies against five zoonotic parasites: *Toxoplasma gondii*, *Entamoeba histolytica*, *Toxocara canis*, *Angiostrongylus cantonensis*, and *Anisakis* species. Tests used were enzyme-linked immunosorbent assays (ELISA), latex agglutination (LA) test, indirect fluorescence antibody (IFA) test, hemagglutination (HA) test, and gel diffusion precipitation (GDP) test. Some 64% of the subjects had antibodies to *T. gondii*. The prevalence of antibodies to *E. histolytica* varied from 2 to 15% depending on the test, but the true rate was probably the 7% or 8% obtained by the HA and IFA tests. The proportions of subjects with positive results were 63% for *T. canis*, 17% for *A. cantonensis*, and 11% for the *Anisakis* spp. The prevalence of antibodies to *T. gondii* and *T. canis* was lower in subjects aged 1 to 9 years than in older subjects, probably because the persons in this group had less time to be infected. Antibody titers to *A. cantonensis* and the *Anisakis* spp. were high in the juvenile group, perhaps because recent changes in eating habits have increased opportunities for infection.

INTRODUCTION

Parasite distribution in Indonesia has been studied by several groups (Bakta *et al*, 1993; Mangali *et al*, 1993; Chomel *et al*, 1993). However, most such studies were based on stool examinations and therefore they cannot provide information on certain protozoa or larva migrans, so that the actual prevalence and route of these infections are not known.

Toxoplasma gondii and *Entamoeba histolytica* are protozoa known for their broad distributions, high prevalence rates, and the severeness of the symptoms they cause in humans. In Indonesia, particularly in Java, dogs are few and many cats are kept as pets instead since more than 90% of the inhabitants are Muslim. The incidence of *T. gondii* is likely to be high in such circumstances because cats are the definitive host for this protozoon. Studies on this protozoon are few (Gandahasada, 1991).

E. histolytica is likely to occur in tropical areas if hygienic conditions are poor. Recent reports have shown that there are many carriers in endemic areas and from the point of view of public health, the disease is an important human infection in tropical countries. Some infected people without symptoms carry cysts.

Toxocara canis, *Angiostrongylus cantonensis*, and *Anisakis* spp. are helminths for which the definitive hosts are dogs, rats, and marine mammals, respectively. Transmission to humans is by the oral intake of embryonated *T. canis* eggs, land snails or slugs in the case of *A. cantonensis*, and marine inhabitants (such as saurel, mackerel, and squid) in the case of *Anisakis* spp. All of these parasites can cause larva migrans, in which symptoms are severe and diagnosis is difficult.

The occurrence of these five parasite infections is closely associated with the eating and other living habits of the inhabitants. Surveys in East Java have been few and the actual state of infection is not known in detail. The purpose of this study was to examine by immunological tests serum samples from inhabitants of Sidoarjo, East Java, Indonesia and to establish the prevalence of antibodies to these five major zoonotic parasites.

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MATERIALS AND METHODS

The survey was done in the 18 months from May 1992 to October 1993. A total of 244 subjects, 124 male and 120 female, were selected from those who visited Sidoarjo City Hospital in East Java; 97 patients complained of diarrhea and the other 147 persons visited the hospital for routine medical examinations and did not have any symptoms. Table 1 shows the sex and age distribution of the subjects; they were from 1 to 80 years, with a mean age of 35 years. At the time of blood collection, the subjects were given a questionnaire for recording of the subject's sex, age, living environment, etc. The questions about their living environment were whether water was piped into the house and whether toilet facilities, either indoor or outdoor, were available.

Table 1
Subjects giving samples, classified by age and sex.

Age (years)	Sex		Total no.	%
	Male	Female		
1-9	17	12	29	12
10-19	11	10	21	9
20-29	18	32	50	20
30-39	25	24	49	20
40-49	12	14	26	11
50-59	21	16	37	15
60-69	10	6	16	7
70-80	10	6	16	7
Total	124	120	244	101

Percents do not total 100 because of rounding off.

Tests

A latex agglutination kit (LA: Toxo-test; Eiken Co Ltd, Tokyo, Japan) was used to examine sera for antibodies to *T. gondii*. The results of a test of serum diluted 32-fold were compared with those on a chart provided by the manufacturer. *E. histolytica* was looked for with the following four tests: an enzyme-linked immunosorbent assay (ELISA), an

indirect fluorescent antibody (IFA) test, a hemagglutination (HA) test (Amoeba HA; Japan Lyophilization Laboratory, Tokyo, Japan), and a gel diffusion precipitation (GDP) test. For examination of *T. canis*, *A. cantonensis*, and the *Anisakis* spp, ELISAs with antigens prepared by the methods described below were done.

Antigens

E. histolytica antigen was prepared from trophozoites (HM-1: IMSS strain; known to have high virulence) that had been cultured in our laboratory under aseptic conditions. The parasites were then suspended in phosphate buffer, sonicated, and used as the antigen at the protein concentration of 5 µg/ml for ELISA, and at 5-10 mg/ml for GDP. In the IFA test, trophozoites (antigen) adhered to the slide glass. In tests for *A. cantonensis*, soluble adult-worm antigen was used at the protein concentration of 5 µg/ml. In tests for *T. canis* and the *Anisakis* spp, third-stage larvae were cultured in Medium 199 (Nissui Pharmaceutical Co, Tokyo, Japan) for 10 to 14 days before their excretory-secretory antigen was used. The protein concentration of this antigen was adjusted to 5 µg/ml.

ELISA

The wells of a microtiter plate were coated with 5 µg/ml antigen. This immunosorbent was first reacted with test sera diluted 1 : 100 and then with anti-human immunoglobulin G (gamma-chain-specific; Tago, Inc, Burlingame, CA) diluted 1 : 1,000. The resulting enzyme activity toward the solid phase was measured with 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid; Sigma, St. Louis, MO). The absorbance at 410 nm (A_{410}) was measured (Uga *et al*, 1990) with a Microelisa Minireader MR 590 (Dynatech Laboratories, Alexandria, VA) and expressed as the ratio, T/N, of the test serum to the mean A_{410} of the negative-control sera. On the basis of the results of preliminary experiments, T/N values of ≥ 3.3 were regarded as showing a positive result, and those of < 1.8 were regarded as showing a negative result. Intermediate results were not obtained.

RESULTS

The prevalence rates of antibodies to four of the

Table 2

Seropositivity for four parasites in different age groups.

Subject's age (years)	Total no. of samples tested	No. (%) of samples giving positive results for:			
		<i>T. gondii</i>	<i>T. canis</i>	<i>A. cantonensis</i>	<i>Anisakis</i> spp
1-9	29	9 (31)	6 (21)	10 (34)	6 (21)
10-19	21	15 (71)	15 (71)	5 (24)	4 (19)
20-29	50	33 (66)	35 (70)	10 (20)	5 (10)
30-39	49	32 (65)	33 (67)	6 (12)	3 (6)
40-49	26	19 (73)	18 (69)	4 (15)	2 (8)
50-59	37	26 (70)	25 (68)	4 (11)	3 (8)
60-69	16	15 (94)	10 (63)	2 (13)	2 (13)
70-80	16	7 (44)	11 (69)	1 (6)	1 (6)
Total	244	156 (64)	153 (63)	42 (17)	26 (11)

parasites in the different age groups are shown in Table 2. Of the 244 subjects, 156 (64%) had antibodies to *T. gondii*. The prevalence rate in the group of subjects aged 1 to 9 years was 30% (9/30), and in older subjects, the rate was generally about 70%, except for a value less than 50% in the group of subjects in their 70s. The prevalence rate of *T. canis* antibodies was 63%. The pattern of seropositivity in different age groups was similar to that of *T. gondii*; the prevalence rate in the subjects aged 1 to 9 years was 20% but the rates in the other groups were higher than the mean value for all patients. Some 17% of the subjects had antibodies for *A. cantonensis*. The prevalence rate tended to be lower in the older age groups; the mean in the subjects aged 1 to 29 years was 25% (25/99) but it was 12% (17/145) for subjects aged 30 years or more. The results obtained with *Anisakis* spp resembled those obtained with *A. cantonensis*; the prevalence rate in the subjects aged 1 to 29 years was 15% (15/99) and that of the subjects aged 30 years or more was 8% (11/145).

Table 3 shows the results for *E. histolytica* antibodies by the four tests used. The highest value, 15%, was obtained by ELISA. The values were 8% by the IFA test, 7% by the HA test, and 2% by the GDP test. There was no sample that gave negative results by ELISA but positive results by other tests. The prevalence rate in the group with diarrhea was slightly higher than that in the group without diarrhea by the three tests other than ELISA. For both groups, the differences in results by the different tests were not significant by the X² test.

Table 4 shows the relationship between the availability of toilet and bathing facilities and the prevalence rates for *E. histolytica* antibodies examined by the IFA test. Of the 244 subjects, 66 (27%) had both kinds of facilities. Ninety-four subjects (39%) had only one kind of facility, and 84 (34%) had neither. The prevalence rate in the subjects with both facilities was higher (9%) than the mean values in other groups (6% and 8%), but the differences were not significant ($p > 0.01$) by the X² test.

DISCUSSION

T. gondii is a protozoon distributed all over the world with a broad host range from mammals to birds and reptiles. Indonesia is no exception; the organism has been found throughout the country (Gandahusada, 1991). Gandahusada describes two seroepidemiological studies carried out in East Java. In one survey, in which the IFA test was used, the prevalence rate was 63%. In the other survey, in which the HA test was used, the prevalence rate was 9%. Thus, the extent of *T. gondii* infection was not clear, but our finding of a prevalence rate of 64% by the IFA test confirmed the findings of the first survey mentioned. Another district in Indonesia, Malili, which is in South Sulawesi (Carney *et al*, 1978), has been found to have a similarly high prevalence (62%). When 50 Japanese subjects whose age and sex were matched to 50 of our subjects in Indonesia were examined to confirm the

Table 3
Seropositivity for *Entamoeba histolytica* by different tests.

Tests	No. (%) of samples giving positive results from:			p*
	Subjects with diarrhea (97)#	Subjects without diarrhea (147)	Total subjects (244)	
ELISA	14 (14)	22 (15)	36 (15)	> 0.05
I FA	9 (9)	10 (7)	19 (8)	> 0.05
HA	9 (9)	9 (6)	18 (7)	> 0.05
GDP	5 (5)	1 (1)	6 (2)	> 0.05

ELISA, Enzyme-linked immunosorbent assay; IFA, indirect fluorescence antibody test; HA, hemagglutination test; GDP, gel diffusion precipitation test.

Figures in parentheses are numbers of samples examined.

* Differences between groups with diarrhea and without diarrhea by the test.

Table 4

Relationship between availability of toilet and bathing facilities (running water), and the finding of *E. histolytica* antibodies by the IFA test.

Plumbing facilities available	No. of samples	No. giving positive results	% of samples in that group
Toilet and running water	66 (27)*	6	9
Toilet	82 (34)	5	6
Running water	12 (5)	1	8
Neither	84 (34)	7	8
Total	244 (100)	19	8

* Percentages of total samples are in parentheses.

reliability of the LA test, the prevalence rate was 24% (unpublished data). This value agreed with that reported in Japan in the past by various tests, so the prevalence rate of 64% in Indonesia seemed to be closed to the real value and not false-positive findings arising from selection of either the test reagent or the technique.

Schantz (1989) included in his review the sero-epidemiological studies of *Toxocara* spp. has been carried out in various part of the world. The prevalence rates depended on the region, ranging from

3% in the United States to 83% in St Lucia. The prevalence rates for the antibodies in Europe, Oceania, and Japan are similar to the rate in the United States, but that the rate is high (Between 50% and 80%) in developing tropical countries such as Colombia, St Lucia, and Taiwan. In our study, the rate for *T. canis* antibodies was 63%, within the reported range. To the best of our knowledge, there has been no survey of *T. canis* infection in Java, but cases have been found in Bali (Chomel *et al*, 1993), about 250 km to the south-east. In that report, 190 serum samples from subjects between 1 and 24 years of age were examined by ELISA, and 63% of samples were positive. It is generally believed that this infection is more frequently to be found in small children than in adults (Schantz, 1989) but such a pattern was not found in our study. Similar results have been obtained in Japan (Uga *et al*, 1990). We used a *T. canis* antigen here, but immunological differentiation between *T. canis* and *T. cati* is difficult and positive findings by this method should be considered as reflecting antibodies to the genus *Toxocara*. Probably one reason for the lower prevalence rate in the subjects aged 70 years or more was that their immune response had declined.

The definitive host of *A. cantonensis* is rodents of the genus *Rattus*, which have been found in Sumatra, Java, and Sulawesi (Stafford *et al*, 1976; Wirereno, 1978). In Semarang, Central Java, in Indonesia, infections in humans have been reported (Widagdo *et al*, 1977). In our study, antigens from the adult worm were used, and cross-reaction with

other nematode was possible; however, *A. cantonensis* may well be found in the district we studied. The tendency for a higher prevalence rate for the antibody in younger subjects probably arose from differences in eating habits; the young people in the district now often eat fresh vegetables (Subagiyo, personal communication) and inadvertent consumption of slugs hidden in the vegetables may occur.

Anisakis spp are found in Indonesia and have been isolated from fish caught in the waters neighboring Indonesia and sold at local fish markets (Ilahude *et al*, 1978; Hadidjaja *et al*, 1978). However, Indonesians do not customarily eat raw fish and, therefore, infection of humans has been thought unlikely; there have been no case reports from Indonesia. Still, 26 (11%) of our 244 subjects were found to have antibodies. The possibility of cross-reactions with antigens of other parasites cannot be ruled out, but four of the 26 positive samples gave values more than twice that of the cut-off value ($A_{410} = 0.27$), suggesting that these results may not have arisen from cross-reactions. The unexpected finding may have occurred because of diversification of eating habits among the young (kinds of fish hitherto unknown are now available in the market; Indah, personal communication).

For diagnosis of *E. histolytica*, serodiagnosis as well as a conventional stool examination is important to help prevent false-negative findings (Patterson *et al*, 1980). In seroepidemiological surveys of *E. histolytica* in various districts of Indonesia in which the indirect hemagglutination test was used, results were positive for 23%, 31% and 18% of samples obtained in South Sulawesi (Carney *et al*, 1978), Sumatra (Cross *et al*, 1976), and Central Java (Cross *et al*, 1975), respectively. In our study, results were positive in 2% to 15% of the samples, depending on the test. The sensitivity of the GDP test and the specificity of ELISA are not entirely satisfactory. The results of the IFA test agreed well with those of the HA test, and the prevalence rate of *E. histolytica* antibodies in this area was probably about 7% or 8%. We do not know why, unexpectedly, no difference was found in the prevalence rate of antibodies in the subjects with and without diarrhea or between those living where toilet and bathing facilities were available and those who were not.

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