

SEROEPIDEMIOLOGIC STUDIES IN OAXACA, MEXICO¹

Search for parasitic antibody using the indirect hemagglutination test

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The indirect hemagglutination test provides an effective serologic method for gaining information on the prevalence of parasitic infections. Data from Oaxaca State on Chagas' disease, toxoplasmosis, amebiasis, malaria, cysticercosis, and Hydatid disease include indications of a high level of antibodies for Chagas' disease in one area and a low level of antibodies for toxoplasmosis throughout the state.

Introduction

This study was undertaken to obtain information about the prevalence of antibodies to seven human parasitic infections in southern Mexico. Sera collected from five areas of the State of Oaxaca were processed in an indirect hemagglutination (IHA) test by microtiter methods with antigenic extracts of the following parasites: *Trypanosoma cruzi*, *Toxoplasma gondii*, *Entamoeba histolytica*, *Plasmodium knowlesi*, *Echinococcus granulosus*, *Cysticercus cellulosae*, and *Leishmania donovani*.

Another objective was to add information on the effectiveness of the IHA test for broad seroepidemiologic surveys. The test has proven sensitive and specific in diagnosing many parasitic infections (1) and has recently been used for epidemiologic purposes to test sera from Brazil (2, 3), the Cape Verde Islands (4), Somaliland (5), Colombia (Kagan, unpublished), Tobago Island (6), and the United States (2, 7). However, its usefulness as an

epidemiologic tool requires further field evaluation.

Materials and Methods

Environmental description

Oaxaca, one of Mexico's southernmost states (Figure 1), has a wide range of topographic and ecologic conditions (8). At latitude 17° North and longitude 96° West, its climate is semitropical and often cool in the highlands, but tropical in the lowlands. Rainfall in the areas studied ranges from 25 to 42 inches annually; maximum precipitation occurs from May through October, and a dry period is characteristic from November to March.

Three lowland tropical zones form part of the state's boundary. On the south, a narrow coastal area of tropical deciduous forest faces the Pacific Ocean; behind this strip of forest lies the Sierra Madre del Sur, which rises to 13,200 feet. On the east, the state is bounded by a lowland area forming part of the Isthmus of Tehuantepec. To the northwest, contiguous with Veracruz State, is a third lowland area which has a heavy rainfall and tropical evergreen forest cover. In the interior of Oaxaca and along its west and northwest borders lies an irregular expanse of mountains up to 10,800 feet high, covered with a temperate pine-oak forest. Within the latter region three valleys converge, forming an inland plateau with an altitude of roughly 4,700 feet.

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Five areas of Oaxaca (Figure 1) were selected as sample sites for the project; two of these were in tropical zones, two in mountains, and one in the central plateau. Table 1 gives the elevation and mean annual rainfall for each area. In area I along the Pacific coast, sera were obtained from the towns of Puerto Escondido, Chila, and San Pedro Mixtepec (each with approximately 3,000 inhabitants), and from nearby villages. In area II at the southern end of the Isthmus of Tehuantepec, sera were obtained from the larger towns of Tehuantepec, Juchitán, and Salina Cruz (all with approximately 20,000 inhabitants), and from the village of El Espinal. In the mountainous area III (Mixteca Alta), the towns and villages of Nochixtlán, Tlaxiaco, Yolomécatl, and Teposcolula were sampled; and in area IV, another mountainous region, Ixtlán de Juárez and Natividad were sampled. In area V, a valley region, sera were obtained from the state capital of Oaxaca City (population 100,000) and from the nearby smaller communities of Zimatlán, Tlacolula, Díaz Ordáz, and Mitla.

Not included in the study were the tropical

zone contiguous with Veracruz State, the high mountain region in the Sierra Madre, and areas inhabited by people who live one day's distance or more from roads (who comprise almost half the state's two million people).

Sampling methods

A total of 613 sera were collected in the five areas during August and September 1969. Tables 1 and 2 indicate the number of persons sampled in each area and their distribution by age and sex. A satisfactory age distribution was obtained within each decade from 10 through 60, but relatively few specimens were obtained from persons outside that age range. Approximately the same age distribution occurred within each area except in area III, where there were proportionally more older persons and fewer persons under the age of 20. For the five areas and each age group, approximately two-thirds of the specimens taken were from males.

Between 17 and 55 blood samples were collected in each town or village. About one-third of the subjects were chance visitors to

FIGURE 1—Five study areas in the State of Oaxaca, Mexico.

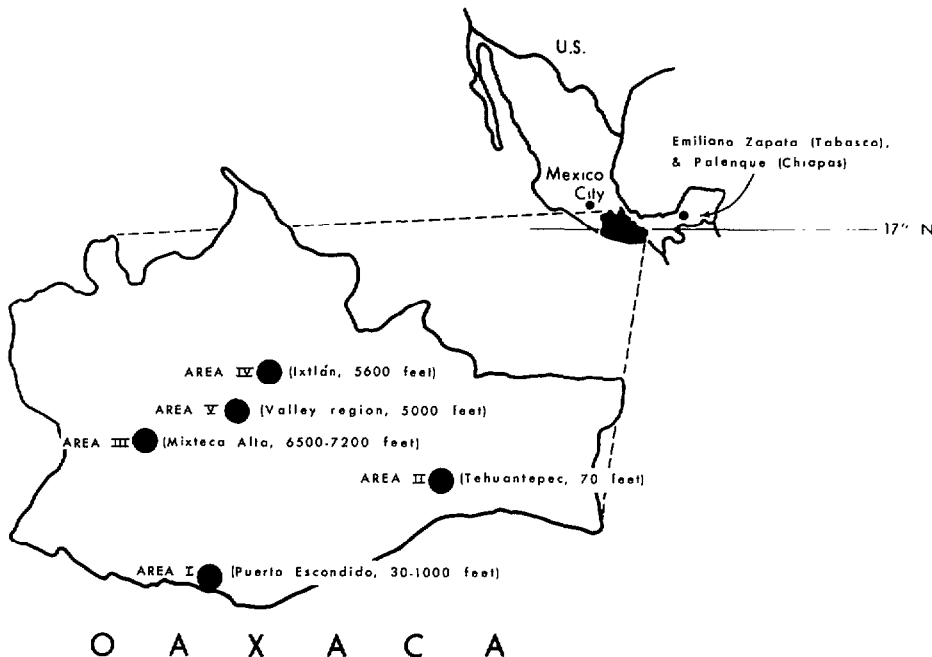


TABLE 1—Percentage of positive reactions in the indirect hemagglutination test with sera from five study areas of Oaxaca using antigens prepared from seven^a parasites.

Study areas in Oaxaca State	No. of sera from each area	Rainfall ^b Altitude Daily mean Temp °C	Percentage of sera with positive hemagglutination titers to the following parasitic antigens:											
			<i>Trypanosoma cruzi</i> (≥ 1:128)		<i>Toxoplasma gondii</i> (≥ 1:256)		<i>Entamoeba histolytica</i> (≥ 1:128)		<i>Plasmodium knowlesi</i> (≥ 1:16)		<i>Echinococcus granulosus</i> (≥ 1:32)		<i>Cysticercus cellulosae</i> (≥ 1:32)	
			No. Pos. ^c No. tested	% Positive	No. Pos. No. tested	% Positive	No. Pos. No. tested	% Positive	No. Pos. No. tested	% Positive	No. Pos. No. tested	% Positive	No. Pos. No. tested	% Positive
Area I (Puerto Escondido and environs)	163	40 in 30-1,000 ft 28°-29°	$\frac{47}{161}$	29	$\frac{2}{159}$	1.2	$\frac{37}{162}$	23	$\frac{93}{159}$	58	$\frac{14}{145}$	9.6	$\frac{9}{159}$	5.6
Area II (Tehuantepec and environs)	137	41 in 70 ft 25°-28°	$\frac{0}{137}$	0	$\frac{18}{137}$	13	$\frac{36}{136}$	26	$\frac{20}{137}$	15	$\frac{7}{137}$	5.1	$\frac{7}{137}$	5.1
Area III (Mixteca Alta)	114	39 in 6,500-7,200 ft 14°-21°	$\frac{3}{114}$	2.6	$\frac{0}{114}$	0	$\frac{31}{105}$	30	$\frac{29}{116}$	25	$\frac{4}{96}$	4.2	$\frac{4}{109}$	3.7
Area IV (Ixtlán)	49	39 in 5,600 ft 13°-18°	$\frac{0}{48}$	0	$\frac{2}{48}$	4.2	$\frac{18}{47}$	38	$\frac{6}{46}$	13	$\frac{1}{49}$	2.0	$\frac{0}{49}$	0
Area V (Valley region)	150	26 in 5,100 ft 17°-23°	$\frac{2}{150}$	1.3	$\frac{5}{150}$	3.3	$\frac{56}{144}$	39	$\frac{38}{150}$	25	$\frac{11}{149}$	7.4	$\frac{3}{149}$	2.0
Total	613		$\frac{52}{610}$	8.5	$\frac{27}{608}$	4.4	$\frac{178}{594}$	30	$\frac{186}{608}$	31	$\frac{37}{576}$	6.4	$\frac{23}{603}$	3.8

^aNone of 610 sera tested with *Leishmania donovani* antigen gave positive reactions (≥ 256).

^bInches/annum.

^cNo. Positive.

TABLE 2—Age and sex distribution of persons providing sera from study areas in Oaxaca, Tabasco, and Chiapas states.

Age group (years)	Sera from areas I-V in Oaxaca			Sera from Emiliano Zapata (Tabasco) and Palenque (Chiapas)		
	Number and percentage of persons in each age group		% Males in each age group	Number and percentage of persons in each age group		% Males in each age group
	No.	%		No.	%	
5-9	16	2.6	63	0	0.0	0
10-14	48	7.8	58	7	10.8	43
15-19	115	18.8	55	8	12.3	50
20-29	213	34.8	66	17	26.2	41
30-39	97	15.8	66	13	20.0	50
40-49	67	10.9	66	11	16.9	18
50 +	57	9.3	67	9	13.8	40
Total	613	100.0	64	65	100.0	42

clinics at a time when the research team was working in the community. Others were called to the clinic by the village president or nurse, or were contacted by house-to-house visit. Men in factories, prisons, and the army provided a third source of sera (about 25 per cent of the total sample). Approximately 75 per cent of the subjects, particularly women and children, had lived in the same area of Oaxaca all their lives. Persons who had lived outside the state for more than 3 months were excluded.

Serologic test procedures

Indirect hemagglutination tests, using tanned red blood cells sensitized with various parasitic antigens, were patterned after the technique of Boyden (9). The following procedure (1) was used with all antigens except the malarial one, for which slight modifications in technique are recommended (10). Fresh sheep red cells were collected aseptically in 8.3% sodium citrate and stored at 4°C. (human Group O cells were used with the malaria antigen). The cells were washed three times with phosphate-buffered saline solution (PBS) at pH 7.2 and adjusted to a 3.8% suspension with PBS. An equal volume of 1:20,000 (weight:volume) tannic acid in PBS was added, and the mixture was incubated at 37°C for 15 minutes. After tanning, the cells were centrifuged, washed once with PBS at pH 7.2, and

resuspended to a 2.5% concentration with PBS at pH 6.4. They were then sensitized by adding an equal volume of a predetermined dilution of antigen, and the mixture was incubated for 15 minutes at 37°C. After centrifugation and removal of the antigen solution, the sensitized cells were washed twice with a 1% solution of normal rabbit serum (NRS) in PBS at pH 7.2; they were then made up to a 1% cell suspension (in 1% NRS) for use in the test.

Serum specimens were inactivated for 30 minutes at 56°C. One per cent NRS (in PBS at pH 7.2) was used as diluent, 0.05 ml being placed in each well of disposable microtitration plates. To the first well we added 0.05 ml of a test serum; then serum dilutions were made, starting at 1:2 and continuing through 1:4,096. One drop (0.025 ml) of the 1% suspension of sensitized cells was added to each well of serum dilution; the plates were shaken, and the cells were allowed to settle. The end point of a positive test was indicated by a mat of cells covering the bottom of the well, equivalent to a 4+ reaction; a reaction was negative when the cells settled to form a compact button or ring at the center of the well.

For the complement fixation test, we employed the method standardized by the U.S. Public Health Service Center for Disease Control for use with bacterial, viral, and parasitic antigens, adapted to a microtechnique. This method requires use of the 50% hemo-

lytic complement technique and precise standardization of all reagents (11). Two control sera from patients with proven cases of the disease in question were included in each serologic test.

Antigens

All antigen solutions used in the serologic tests were soluble extracts of the parasitic organisms; the sole exception was the antigen solution of *Echinococcus*, for which we used cystic fluid. A brief description of each antigen preparation is given below:

Hemoflagellates—saline extracts of cultured *T. cruzi* and *L. donovani* organisms delipidized with benzene before extraction (1, 12).

Toxoplasma—a crude water-soluble extract of *T. gondii* organisms collected from infected mouse peritoneal fluid (1, 13).

Amoeba—a crude extract of cultured *E. histolytica* organisms disrupted by sonification (1, 14).

Plasmodia—a crude saline extract of *P. knowlesi* organisms collected from infected monkey blood (1, 10).

Taenia—a saline extract of *C. cellulosae* cysts treated with acetone before extraction (1).

Echinococcus—concentrated hydatid fluid from *E. granulosus* cysts of infected sheep (1, 15).

Results

Trypanosoma cruzi

In the IHA test, forty-seven of 161 sera (29%) from area I of Oaxaca had positive reactions with an extract of *T. cruzi* antigen. This contrasted with low rates (0 to 2.6%) in the other areas of the state (Table 1). Within area I, sera from 17 of 38 persons (45%) from Chila were positive, as were sera from 4 of 55 persons (7%) from Puerto Escondido, and those from 25 of 68 persons (37%) from other nearby towns—including San Pedro Mixtepec. We noted no sex differences in the distribution of hemagglutination titers in area I. When the data from area I (excluding the town of Puerto

Escondido) were analyzed by age, the percentage of positive reactions increased progressively in each decade of life (see Table 3).

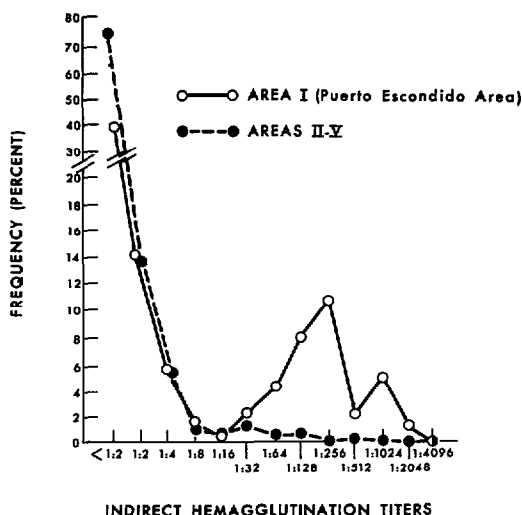
TABLE 3—Age distribution of persons from area I of Oaxaca^a (Puerto Escondido region) whose sera reacted positively to an indirect hemagglutination test using an antigen prepared from *Trypanosoma cruzi*.

Age group (years)	Number tested	Number positive	% positive ($\geq 1:128$)
5-9	2	1	—
10-19	23	7	30
20-29	31	12	39
30-39	24	9	38
40-49	15	7	47
50+	11	7	64
Total	106	43	41

^aThe tabulation includes all sera tested from Area I except 54 from the town of Puerto Escondido, where only 7% of the sera tested were positive.

The frequency distribution of hemagglutination titers in sera from area I resulted in a trimodal curve, with peaks at titers of 1:256 and 1:1,024; the distribution of titers from area II to V resulted in a curve without a significant second peak (Figure 2). These curves, plus previous experience with the IHA test for *T.*

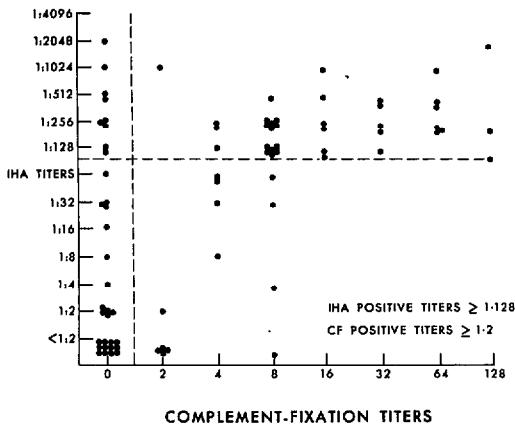
FIGURE 2—Frequency distribution of titers obtained in the indirect hemagglutination test with sera from Oaxaca State, using an antigen prepared from *Trypanosoma cruzi*.



cruzi (2; Kagan, unpublished), led us to consider titers of 1:128 and higher as positive.

Figure 3 shows the results of tests establishing the correlation between IHA and complement fixation (CF) titers. The tests were carried out on 83 sera, including most of those showing positive reactions by IHA test and a random group of those reacting at nonsignificant titers. Thirty-seven sera gave positive reactions for Chagas antibodies in both the IHA test (1:128 or higher) and the CF test (1:2 or higher), and 24 registered negative in both tests; thus there was 74 per cent agreement between the tests. Of the remaining sera, 13 were positive by CF but negative by IHA, and 9 were negative by CF but positive by IHA.

FIGURE 3—Correlation of indirect hemagglutination and complement fixation titers for 83 Oaxaca sera tested with antigens prepared from *Trypanosoma cruzi*.



Toxoplasma gondii

Of 608 Oaxaca sera tested for toxoplasmosis antibodies, 27 (4.4%) had positive titers. Guided by previous experience with the IHA test for titrating antibodies to *T. gondii* antigen, we considered a titer of 1:256 to be a positive reaction (3, 7). Prevalence rates, by area of the state, ranged from no positive reactors in the mountainous area III (Mixteca Alta) to 13 % positive in the coastal area II (Tehuantepec—see Table 1). Prevalences for different age groups are shown in Table 4.

Sixty-five additional sera (shown in Table 2)

TABLE 4—Age distribution of persons from Oaxaca, Tabasco, and Chiapas whose sera reacted positively to an indirect hemagglutination test using an antigen prepared from *Toxoplasma gondii*.

Age group (years)	Sera from areas I-V in Oaxaca		Sera from Emiliano Zapata (Tabasco) and Palenque (Chiapas)	
	No. tested	% Positive ($\geq 1:256$)	No. tested	% Positive ($\geq 1:256$)
5-9	19	0	0	0
10-14	48	8.3	7	14
15-19	115	5.2	8	25
20-29	210	4.3	17	35
30-39	96	5.7	13	31
40-49	64	4.7	11	36
50+	56	0	9	33
Total	608	4.4	65	31

were included in the toxoplasmosis test. Forty of these came from Emiliano Zapata (population 3,600) in the State of Tabasco and 25 came from a rural area near Palenque in the State of Chiapas. Both towns are near the Gulf Coast of southern Mexico in a tropical rain forest area (having an altitude of approximately 300 feet and a rainfall of about 125 inches per year). Thirty-one per cent of these sera gave positive reactions. Although the Tabasco-Chiapas sample was small, the age distribution compares favorably with that of the Oaxaca sample (Table 2). A frequency distribution curve based on the serologic titers of the Oaxaca sera showed no clear modality; but the sera from Tabasco and Chiapas States produced a unimodal curve that reached a maximum titer at 1:64 (Figure 4). Neither group of sera showed significant differences in prevalence rates by age or sex.

Entamoeba histolytica

Of the 594 Oaxaca sera processed in the IHA test with an extract of *E. histolytica* used as the antigen, 178 (30%) gave positive results; the percentage of positive test subjects in each of the five study areas ranged from 23 to 39% (Table 1). The frequency distribution of serologic reactions resulted in a unimodal curve

FIGURE 4—Frequency distribution of indirect hemagglutination titers for sera from Oaxaca, Tabasco, and Chiapas states tested with an antigen prepared from *Toxoplasma gondii*.

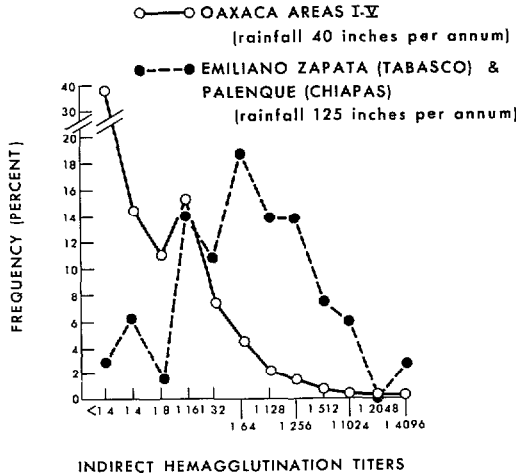
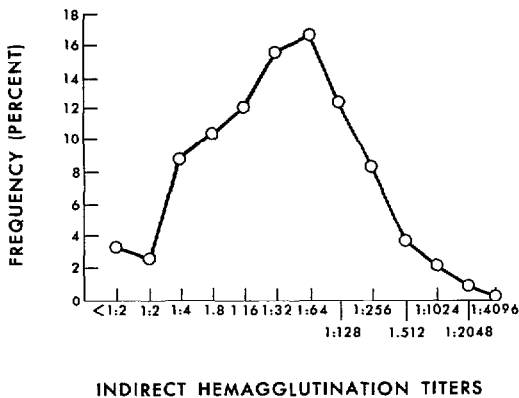


TABLE 5—Age distribution of persons from the five study areas of Oaxaca whose sera reacted positively to an indirect hemagglutination test using antigens prepared from *Entamoeba histolytica* and *Plasmodium knowlesi*.

Age group (years)	<i>Entamoeba histolytica</i>		<i>Plasmodium knowlesi</i>	
	No. tested	% Positive (≥ 1:128)	No. tested	% Positive (≥ 1:16)
5-9	16	19	18	0
10-14	48	27	36	9
15-19	110	35	125	22
20-29	209	33	212	31
30-39	96	26	97	38
40-49	65	22	66	48
50+	50	34	54	37
Total	594	30	608	31

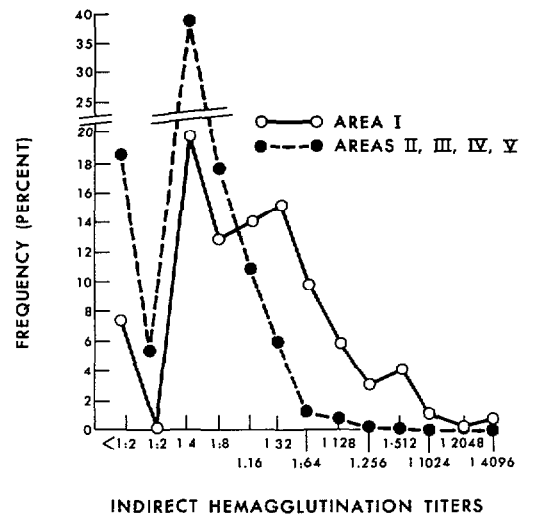
FIGURE 5—Frequency distribution of indirect hemagglutination titers for Oaxaca sera tested with an antigen prepared from *Entamoeba histolytica*.



antigen. By contrast, a seropositive rate of 13 to 25% was found in the other areas of the state (Table 1). Calculation of antibody prevalence in each area by geometric mean titers gave the following results: area I, 12.8; II, 3.0; III, 4.1; IV, 2.7; and V, 3.8.

Figure 6 shows frequency distribution curves based on serologic titers of 608 Oaxaca sera. A curve for area I, where malaria transmission is continuing, reached a peak at the

FIGURE 6—Frequency distribution of indirect hemagglutination titers for Oaxaca sera tested with an antigen prepared from *Plasmodium knowlesi*.



peaking at 1:64 (Figure 5). This curve and previous experience with the test (14) led us to regard a titer of 1:128 or higher as positive. No significant age or sex differences appeared in the distribution of titers (Table 5).

Plasmodium knowlesi

In area I, 93 of 159 sera tested (58%) had positive IHA titers (1:16 or over) when an extract of *P. knowlesi* was used as the test

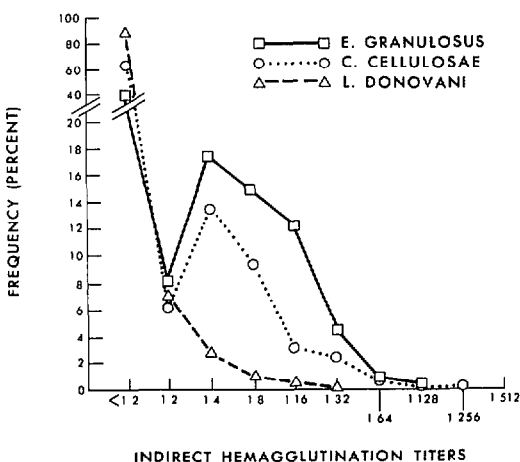
nonspecific titer of 1:4, but tended toward a bimodal shape with a second peak at 1:32. A separate curve for areas II to V, zones where malaria transmission is no longer thought to occur, also reached a maximum titer at 1:4, but then decreased more rapidly to the base line.

The distribution of ten-year age groups' seropositive reactions for the five areas showed a gradual increase in the percentage of positive reactors for each group through ages 40-49, followed by a reduction in the rate for older groups (Table 5). Distribution of positive reactions by sex showed that 35% of the men and 26% of the women were seropositive.

Echinococcus granulosus and *Cysticercus cellulosae*

When tests are conducted with microtiter methods at the Center for Disease Control, a positive titer for antigenic extracts of both these organisms is considered to be 1:32 or higher. Of 603 sera tested with *C. cellulosae* antigen, 3.8% reacted positively, as did 6.4% of 576 sera tested with *E. granulosus* antigen (Table 1). Figure 7 shows frequency distribution curves of serologic reactions to these antigens, and Table 1 shows the distribution of seropositive reactions by area.

FIGURE 7—Frequency distribution of indirect hemagglutination titers for Oaxaca sera tested with antigens prepared from *Leishmania donovani*, *Echinococcus granulosus*, and *Cysticercus cellulosae*.



Leishmania donovani

None of the sera reacted positively with *L. donovani* antigen (a titer of 1:256 or higher was considered positive).

Heterophile antibody

All sera were tested for heterophile antibody; 83% had titers of less than 1:2, 15% had titers of 1:2, and 2% had titers of 1:4. Because of these low reactions, none of the sera were absorbed to remove the heterophile antibody.

Discussion

With improvements in the quality of parasitic antigens and advances in serologic methods, three tests have been of particular value in determining prevalence rates for parasitic antibodies in epidemiologic studies. These are the complement fixation, indirect fluorescent antibody and indirect hemagglutination (IHA) tests. The IHA test has become the main seroepidemiologic method for parasitic studies at the Center for Disease Control laboratory—because of its sensitivity, specificity, and reproducibility with many antigens, and because of the ease with which a large number of samples can be processed with microtiter methods (1). In this study and in several earlier studies (2-7) it has been used to determine the prevalence of parasitoses in a community. The IHA test's usefulness in surveillance of malaria eradication programs is also being tested (6, 16).

When a large collection of sera from an endemic area is titrated for a parasitic antibody by the IHA method, the subsequent plotting of frequencies (on the ordinate) and titers (on the abscissa) often produces a bimodal curve. The first mode, which occurs in the portion of the curve with low titers, represents nonspecific reactions obtained from noninfected persons or from members of the population with low antibody titers. The second mode represents titers from persons with specific antibody for the antigen employed. We found examples of this bimodal distribution for *T. cruzi*, *E. granu-*

losus, and *C. cellulosa* antigens (Figures 2 and 7). The shape of the frequency distribution curve provides a way to establish a diagnostic titer for a particular laboratory test, because a positive titer for a parasitic antigen usually begins about where the ascending slope of the second mode increases.

Chagas' disease

The 29% prevalence of *T. cruzi* antibody found in sera from area I by IHA test compares with prevalences of 7 to 13% by CF test reported from the three Mexican states of Michoacán, Guerrero, and Zacatecas (17, 18, 19, 20). The Oaxaca findings confirm the prediction of Tay et al. (21) that Pacific coastal areas of the state might have zones of high endemicity.

Chagas' disease is not diagnosed clinically in Oaxaca at present, but the first two cases reported from Mexico in 1940 (22) were from that state. A total of 72 cases have since been reported from the entire country (23, 24); some were diagnosed parasitologically by finding *T. cruzi* in the blood, others by positive CF tests, and two by finding leishmania forms of *T. cruzi* in microscopic sections of the heart at autopsy (25). *T. cruzi* has also been found in a variety of nonhuman vertebrate hosts in Mexico, and in at least nine triatomid species, including the following in Oaxaca: *Triatoma dimidiata*, *T. phyllosoma*, *T. barberi* and *Rhodnius prolixus* (21, 24, 26).

For diagnosis and seroepidemiologic study of Chagas' disease, the CF test is the most widely used and best evaluated procedure. In recent years use of the indirect fluorescent antibody and IHA tests has also increased; the latter was used in surveys reported from Brazil (2) and Colombia (Kagan, unpublished). Both tests are as sensitive as the CF test or more so, but the IHA test is believed to give a larger (though still small) percentage of false positive reactions (1, 27, 28). This may explain in part the lack of correlation between some IHA and CF titers in this study (Figure 3), although 74% of the 83 sera tested were in agreement.

One challenging feature of Chagas' disease is the possible existence of several strains of *T. cruzi*, which could be responsible for variations in the disease's severity in different parts of the world or even different parts of a single country. This may explain the infrequent diagnosis of severe Chagas' disease in Mexico. A study is in progress to confirm our seropositive findings by isolating *T. cruzi* from positive subjects, and to determine the degree of pathology such infections produce.

Toxoplasmosis

The low toxoplasmosis prevalence rate (4.4%) obtained in Oaxaca, including the absence of positive reactors in area III, is among the lowest reported throughout the world (29). Higher rates have been reported from neighboring Central American countries, such as Honduras (64%), Panama (41%), El Salvador (68%), and Guatemala (50 to 94%) (30, 31, 32, 33). Previous serologic surveys in Mexico using the methylene blue dye test (34) indicated positive reactions in 19% of 577 persons tested in the State of Oaxaca (from the cities of Oaxaca and Tuxtepec), compared to positive reactions of between 24 and 40% in other states. Toxoplasmosis surveys by skin test in Mexico have revealed positive results ranging from 15 to 57% (35, 36, 37).

We have no explanation for the low prevalence rate obtained throughout Oaxaca, but the finding warrants further investigation. No important differences were noted in prevalence rates of the various areas within Oaxaca, except for the relatively higher rate (13%) found in area II (Tehuantepec). In sharp contrast to Oaxaca, we found a 31% prevalence rate for the two study areas in Tabasco and Chiapas States (both are in a zone of Mexico that has approximately three times more rainfall than Oaxaca). Variations in toxoplasmosis prevalence associated with climate and altitude have been reported by others, suggesting that the disease is transmitted more readily in warm, humid climates and at low altitudes (3, 7, 30, 31, 32, 33, 38).

The IHA test, a sensitive and specific test for

toxoplasmosis, is slightly more reactive than the methylene blue dye test, but is simpler and safer to use (1, 13, 39). It is less efficient than the dye or indirect fluorescent antibody tests in detecting antibodies at the acute stage of infections, but there is good agreement between the tests in diagnosing of chronic infections (1, 3, 40). Single IHA titers higher than 1:256 indicate current or recent past infection with the parasite and may be clinically important. Single titers between 1:64 and 1:256 are of questionable clinical significance for diagnostic purposes, but are epidemiologically important as a sign of probable past infection. The IHA test has been used in several seroepidemiologic studies for toxoplasmosis (3, 4, 5, 7).

Amebiasis

Amebiasis is highly prevalent in Mexico and the disease is often severe. A 1960 report on 89,000 fecal examinations, conducted in many parts of the country during the preceding 25 years, cited positive amebiasis rates ranging from 14 to 74% (41). It also cited a positive rate of 73% for examinations of 40 persons from Oaxaca. The seropositive rate now reported from Oaxaca thus correlates well with the disease's known prevalence in Mexico.

The complement fixation test, the oldest serologic test for amebiasis, has been superseded by other methods, particularly the IHA, gel diffusion, and indirect fluorescent antibody tests (1). The IHA technique is a sensitive and specific indicator of invasive amebiasis (bowel wall or extraintestinal infection) but not a sensitive detector of noninvasive intestinal infections. False negatives occur in less than 1% of hepatic abscesses, and in up to 13% of invasive bowel infections. In noninvasive intestinal amebiasis there is practically no antibody response. False positive reactions range from 0 to 1%. The persistence of antibody after infections have been treated is not well established, but low positive IHA titers can be found in many patients for a year or longer (14).

The IHA test was used for seroepidemiologic investigations in five previous studies (2, 4, 5,

42; Kagan, unpublished). In three of these (2, 4, 5) the percentage of positive reactions was low (2 to 12%) and the resultant frequency distribution curves were bimodal. This study (with 30% positive reactions) and the two other studies (42; Kagan, unpublished) that recorded high seropositive rates (34 and 96%) produced unimodal curves.

Malaria

The Mexican national malaria eradication program has operated in the State of Oaxaca at least since 1956, and is considered to have succeeded in most of the state. Nevertheless, area I has been described as one of the most difficult Mexican regions for achieving eradication, and transmission was continuing when the area was intensely studied in 1962 (43). Clinical evidence and blood film surveys indicate that it still continues. *P. vivax* is the predominant malarial species, but cases of *P. falciparum* do occur. During the 1962 study rare cases of *P. malariae* were also noted (43). The serologic data now reported confirm that transmission is slight or nonexistent in areas II to V, but continues in area I.

The indirect fluorescent antibody (IFA) and IHA tests, the most frequently used serologic methods for malaria detection, are sensitive and specific (1, 16, 44). The IFA test has been used more often in diagnosis, but both tests have been used epidemiologically (6, 45, 46). Technical difficulties limit the use of the IFA test for large-scale studies, but the IHA test can be used with relative simplicity to process many specimens. Because the major deficiency of the IHA test is the antigen's lability, careful control of test conditions is essential.

In seroepidemiologic studies of malaria antibody, prevalence is often determined by two criteria: the percentage of the population with positive titers and the geometric mean reciprocal titer (GMRT).

At the Center for Disease Control a IHA titer of 1:16 or higher is generally regarded as positive. The dynamics of IHA antibody persistence over long time periods have not been

well established. But in regions of high malarial endemicity (where people have had prolonged infections and multiple attacks) titers from some persons apparently remain positive at least 14 years after radical cure and interruption of disease transmission. GMRT prevalences, which may reach levels of 100 or higher in holoendemic areas, fall gradually after malaria transmission stops (6).

Hydatid disease and cysticercosis

The absence of strong positive reactions to *E. granulosus* antigen in this study is consistent with the few reports of autochthonous hydatid disease cases in Mexico (47) and the relatively low incidence of cysts found in animal surveys (48). However, infection with *Taenia solium* tapeworms is common in Oaxaca and Mexico generally, and cysticercosis is a public health problem of considerable magnitude (49, 50). The percentage of positive Oaxaca sera reactions to *C. cellulosae* is in accord with the prevalence of cysticercosis reported from other parts of the country; in Mexico City 97 human cases (3.5%) were found in 2,767 autopsies conducted between 1954 and 1959 (51). A survey of hogs in 17 states in 1954 (52) and in San Luis Potosí in 1966 (53) showed 4.6% to be infected with *C. cellulosae*.

The IHA test for hydatid disease is both sensitive and specific when *E. granulosus* is used as antigen (1, 54), but the parameters of the test for cysticercosis when *C. cellulosae* or *Taenia saginata* is used require further evaluation (1, 50, 55). When using the microtiter technique a titer of 1:32 is currently considered positive for both *C. cellulosae* and *E. granulosus*, but the two antigens frequently cross-react. Still, diagnosis of an *echinococcus* infection is usually possible when *E. granulosus* antigen is used, because the serologic response to a hydatid cyst generally results in high hemagglutination titers plus an associated positive reaction (1:5 or higher) to the bentonite flocculation test. *Cysticercus* antisera cross-react with *E. granulosus* antigen but usually only at low titers. In the Oaxaca

samples both cysticercosis and *E. granulosus* titers were low and of the same magnitude. We therefore believe that the *E. granulosus* response is a cross-reaction to the cysticercosis antibody.

Leishmaniasis

In Mexico the form of dermal leishmaniasis known as Chiclero's ulcer of the ear is caused by *L. mexicana*. The condition is found in the humid lowland areas of southern Mexico in states bordering the Gulf. It has also been reported from the eastern and northeastern lowlands of Oaxaca State (56, 57). Four cases of kala-azar in Mexico have also been described (58, 59). Our IHA tests' failure to detect circulating antibodies is not surprising, first because present methods are unable to detect low levels of antibody from past *L. mexicana* infections, and second because the sample was probably not collected in areas of Oaxaca where the parasite is transmitted. Although *T. cruzi* and leishmanial antigens often cross-react in the IHA test (1, 60), none of our 52 sera positive to *T. cruzi* antigen reacted positively with *L. donovani* antigen.

No routine, commonly accepted, diagnostic serologic test exists for leishmaniasis. Antibody titers are usually low in patients with a present or past history of the self-limiting form of cutaneous leishmaniasis, but sera from individuals with mucocutaneous leishmaniasis or kala-azar have a moderate amount of reactivity. The complement fixation test, used with an extract of mycobacteria as antigen, is the most reliable test for kala-azar. The indirect fluorescent antibody (61) and IHA tests require further evaluation for diagnostic and epidemiologic purposes.

Summary

The indirect hemagglutination (IHA) test is an effective serologic technique for determining the prevalence and distribution of parasitic disease. It was used in Oaxaca State, Mexico, to increase available information on the prevalence

of antibodies for seven human parasitic infections. A total of 613 sera were collected from five study areas: the two tropical lowland areas near Puerto Escondido and Tehuantepec along the Pacific coast, the two mountainous areas of Mixteca Alta and Ixtlán above 5,600 feet, and the interior valley at 5,100 feet.

Antibody prevalence was high when an antigen prepared from *Trypanosoma cruzi* was used in the Puerto Escondido area; 29% of all sera from the area had positive titers, as did those from 17 of 38 persons in one village. An antigen derived from *Toxoplasma gondii* produced very few positive reactions (4.4%);

the range was from none of 114 sera in the Mixteca Alta region to 13% of 137 sera in the Tehuantepec area.

An antigenic extract of *Plasmodium knowlesi* produced a positive reaction for malaria in sera from 58% of persons tested in the Puerto Escondido area and 13 to 25% of those tested elsewhere. Use of *Entamoeba histolytica* antigen resulted in high positive rates (from 23 to 39%) in all areas. All sera reacted negatively to *Leishmania donovani* antigen, but 3.8% responded positively to *Cysticercus cellulosae* antigen, and 6.4% were positive with *Echinococcus granulosus* antigen.

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