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Seroepidemiological Studies by Indirect Haemagglutination Tests for Malaria

Military Recruit Collections from Argentina, Brazil, Colombia, and the United States of America *

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Seroepidemiological studies of 10 956 sera of military recruits from 4 countries in the Western Hemisphere suggest that the indirect haemagglutination test employing an antigen prepared from Plasmodium knowlesi *adsorbed to human group O erythrocytes may be useful in epidemiological studies of malaria. Based on a titre of 1 : 8, or higher, in a microtitration of serum, the prevalence of serological reactors in the collection of sera from USA recruits was 0.9%, in the Argentina collection 4.6%, in the Brazil collection 20.8%, and in the Colombia collection 21.3%. In Brazil and Colombia the distribution of serological reactors correlated with the distribution of malaria as determined by active and passive surveillance. In Argentina, serological reactors were found in states where active and passive surveillance indicated that malaria had been eradicated years ago.*

Reference Room 1666 NS

Epidemiological studies for the prevalence of malaria are being made by the classical methods of fever surveys, spleen-index surveys, or blood-slide examination for parasites. At the present time the indirect fluorescent antibody (IFA) and indirect haemagglutination (IHA) tests hold much promise as serological tools for evaluating plasmodial antibody in individuals and in populations. The IFA test is admirably suited for diagnostic purposes but, until the technique can be simplified or mechanized, it cannot be seriously considered for large-scale epidemiological studies. The IHA test, used for field studies by Desowitz & Saave (1965) in Australia and New Guinea, showed promise as an epidemiological tool. In our laboratory the variables associated with the indirect haemagglutination technique were carefully examined, and a reproducible test of high sensitivity and specificity was developed (Rogers, Fried & Kagan, 1968). This technique employed a soluble extract of *Plasmodium knowlesi* adsorbed to human group O red cells. The antigen is prepared by using a French press essentially as described by Mahoney, Redington & Schoenbechler (1966).

In the present study, this method was used to evaluate malaria antibody in 4 collections of sera obtained from military recruits in the Western Hemisphere. The test was shown to be feasible for epidemiological studies.

MATERIALS AND METHODS

Antigen preparation

Splenectomized rhesus monkeys were infected with the *Anopheles hackeri* strain of *P. knowlesi*[5] by blood transfer. The animals were anaesthetized

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and exsanguinated when 10% or more of their red blood cells contained mature schizonts. Either heparin or sodium citrate was used as an anticoagulant. All further procedures were carried out at 4°C.

The blood cells were washed 3 times in 10 volumes of 0.85% saline solution by centrifuging at 1100 *g* for 5 minutes and lysed by adding at least 10 volumes of distilled water. After the mixture had been centrifuged at 3000 *g* for 5 minutes, the sediment consisted of plasmodia, leucocyte nuclei, and some red-cell stroma. Washing the sediment 3 times with saline solution facilitated removal of most of the fluffy white stroma. The final sediment was stored at −70°C before further processing. Blood from a monkey weighing 3 kg could be processed to yield 5 ml–20 ml of plasmodial sediment in 4 hours.

For further preparation, the plasmodia were thawed in tepid water and placed in a cooled French pressure cell.[1] Two volumes of antigen diluent (equal volumes of 4% NaCl and M/15 phosphate buffer at pH 6.4) were then added. The cell was operated at 20 000 lbf/in² (1406 kgf/cm²). The antigen diluent used for rinsing the cell brought the final volume to 4 times the volume of the plasmodial sediment. The disrupted material was centrifuged at 8000 *g* for 15 min. The colour of the supernatant was tan to pink, and it was opalescent; the residue was black and appeared to consist of malaria pigment and amorphous debris. The supernatant was considered to be undiluted antigen and was used as such, or, when titration tests showed that dilution was compatible with suitable cell sensitivity, it was diluted with antigen diluent. The undiluted antigen was stored at −70°C.

Test procedure

Human group O blood, either fresh or stored for less than 21 days in acid-citrate-dextrose at 4°C, was washed 3 times with phosphate-buffered saline (PBS) solution (equal volumes of 0.85% NaCl and M/15 phosphate buffer at pH 7.2). The buffy coat was discarded. The erythrocytes were suspended in 40 volumes of PBS, and an equal volume of 1 : 20 000 (w/v) tannic acid in PBS was added. The mixture was incubated for 15 min in an ice-bath. After the cells had been washed twice with PBS, they were ready for sensitizing.

[1] American Instrument Company, Inc., Silver Spring, Md., USA. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the US Department of Health, Education, and Welfare.

The packed tanned cells were sensitized by adding 5 volumes of antigen, mixing, and incubating for 15 min at 37°C. To decrease non-specific agglutination, an equal volume of PBS with 5% normal rabbit serum (NRS) was added, and this mixture was incubated for an additional 5 min. After 1 wash in PBS containing 1% NRS at pH 7.2, the cells were suspended in 50 volumes of this solution and used immediately. Cells with 1 : 10 000 thiomersal were useful for 1 week if stored at 4°C. Before stored cells were used, they were resuspended in fresh PBS with 1% NRS.

Plastic plates[2] with an 8 × 12 array of wells were used. Each well first received 0.05 ml of PBS with 1% NRS. A 0.05-ml diluting loop filled with test serum was placed in the first well in each row, and the serum was serially diluted in 2-fold dilution, beginning with a 1 : 2 dilution. One collection of sera (Argentina) was titrated in 3-fold steps at a starting dilution of 1 : 3.

During agitation, 0.025 ml of the 2% sensitized-cell suspension was dropped into each test well. Positive and negative control sera were titrated each day. The completed test was read 45 min–90 min later. A smooth mat of agglutinated cells, a [illegible] reading according to Stavitsky (1954), was considered a positive reaction. In order to detect non-specific agglutination reactions, positive sera were tested with a 2% suspension of tanned red blood cells sensitized with 5% NRS.

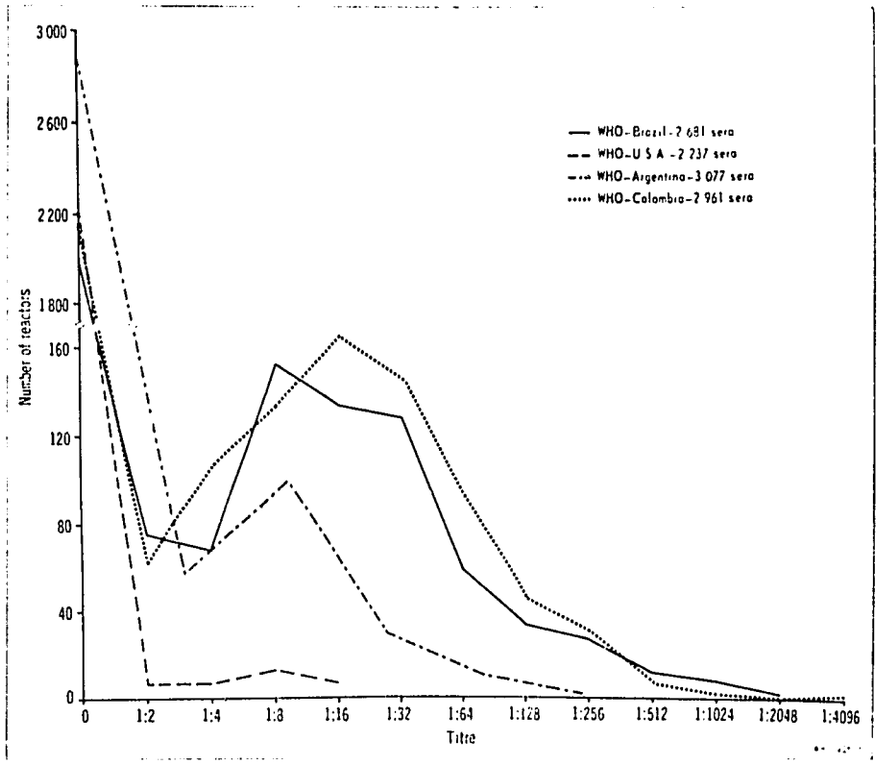
Sera titrated

The sera tested were collected on 4 occasions by the WHO Reference Serum Bank in New Haven, Conn., USA, from male military recruits. A total of 10 956 sera was evaluated: 3077 sera from Argentina; 2681 sera from Brazil; 2961 sera from Colombia; and 2237 sera from the United States of America. The USA collection was described by Paul et al. (1964), and the Brazil collection by Florey et al. (1967). The Colombia and Argentina collections were described by Evans et al. (1969). In general, young men were bled during the months of June and July at the time they were inducted into the army. The sample was designed to include army camps from all areas of the countries. Some of these collections have been extensively studied for other parasitic antibodies (Feldman, 1965; Cuadrado & Kagan, 1967; Walls & Kagan, 1967; Walls, Kagan & Turner, 1967; Lamb & Feldman, 1968).

[2] Cooke Engineering Company, Alexandria, Va., USA.

FIG. 1

FREQUENCY DISTRIBUTION OF TITRES OBTAINED IN THE PASSIVE HAEMAGGLUTINATION TEST WITH SERA FROM MILITARY RECRUITS WITH AN ANTIGEN PREPARED FROM *PLASMODIUM KNOWLESI*



RESULTS

The frequency distribution of titres obtained in the IHA tests with the 4 collections is shown in Fig. 1, and the titres obtained are shown in Table 1. Based on the peak of the specific portion of the curves, a titre of 1 : 8 or greater was considered positive for epidemiological purposes. Using this

TABLE 1

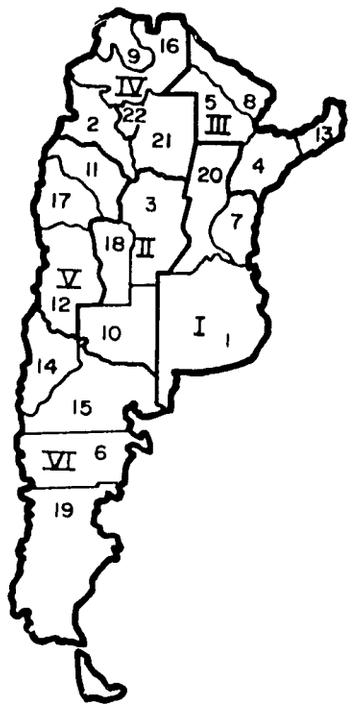
DISTRIBUTION OF INDIRECT HAEMAGGLUTINATION TITRES FOR MALARIAL ANTIBODY OBTAINED WITH MILITARY-RECRUIT SERA

Country	Total no. tested	Reciprocal titres [a]																	
		0	2	3	4	8	9	16	27	32	64	81	128	243	256	512	1 024	2 048	4 096
USA	2 237	2 203	7	—	7	13	—	7											
Brazil	2 681	1 979	76	—	68	153	—	134	—	129	59	—	34	—	27	12	8	2	
Colombia	2 961	2 162	62	—	108	134	—	166	—	146	93	—	46	—	31	8	2	1	2
Argentina	3 077	2 877		58			100		30			10		2					

[a] Dilution schema.

FIG. 2
GEOGRAPHICAL DISTRIBUTION OF THE ARGENTINIAN RECRUITS

FIG. 3
STATES AND PUBLIC HEALTH AREAS IN ARGENTINA [a]



[a] Roman numerals (I–VI) refer to public health areas, each comprised of more than 1 state. Small numbers refer to the individual states listed in Tables 2, 3 and 5.

criterion, the following prevalence of positive reactors was obtained: USA, 20 out of 2237 (0.9%) specimens positive; Argentina, 142 out of 3077 (4.6%) specimens positive; Brazil, 558 out of 2681 (20.8%) specimens positive; and Colombia, 629 of 2961 (21.3%) specimens positive.

Argentina

The geographical distribution of the recruits sampled is shown in Fig. 2. The greatest concentration occurred in the north-eastern part of the country, intensifying toward Buenos Aires. The public health areas are shown in Fig. 3. Table 2 shows the number of positive serological reactors for each state within the 6 public health areas. Areas II and V had the highest serological prevalence rates. The prevalence of antibodies varied from state to state, ranging from zero in 7 states to a maximum of 28% serological positive in the State of Tucumán. The sample for Tucumán (50 sera) was, however, small.

Table 3 shows the number of slide-positive malaria cases in selected states from 1962 to 1965. These figures were obtained from active and passive surveillance records. These rates also show marked variation, ranging from zero positive in Catamarca to 11% positive in Chaco.

Fig. 4 shows the geographical distribution of reactors obtained by slide and serological methods. The discrepancy in the distributions will be discussed later.

Table 4 shows the distribution of positive reactors among blood groups in the ABO system. The distribution of positive serological reactors in the A, B, and O subgroups, respectively, was not significantly

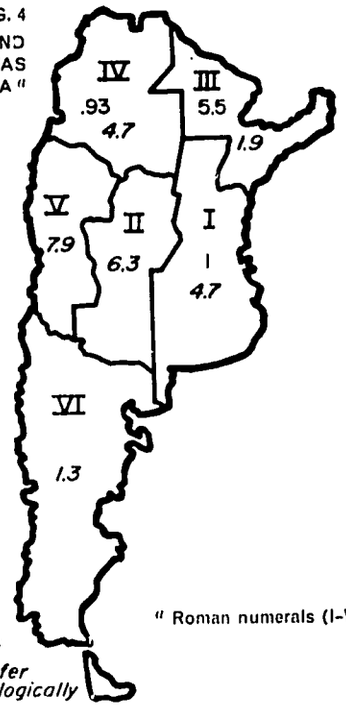
TABLE 2

ARGENTINA: THE NUMBER OF POSITIVE SEROLOGICAL REACTORS TO A *PLASMODIUM KNOWLESI* ANTIGEN FOR MALARIA ANTIBODY BY STATE AND PUBLIC HEALTH AREA

State code	Name of state	Public health area	No. positive	No. tested	% positive
1	Buenos Aires	I	53	1 147	4.6
7	Entre Rios		9	154	5.8
20	Santa Fe		7	155	4.5
	Total for area I		69	1 456	4.7
3	Córdoba	II	22	236	9.3
10	La Pampa		5	183	2.7
18	San Luis		3	60	5.0
	Total for area II		30	479	6.3
4	Corrientes	III	1	71	1.4
5	Chaco		0	38	0
8	Formosa		0	3	0
13	Misiones		2	45	4.4
	Total for area III		3	157	1.9
2	Catamarca	IV	2	94	2.1
9	Jujuy		1	64	1.6
16	Salta		10	186	5.4
21	Santiago del Estero		0	177	0
22	Tucumán		14	50	28.0
	Total for area IV		27	571	4.7
11	La Rioja	V	0	6	0
12	Mendoza		9	84	10.7
17	San Juan		0	24	0
	Total for area V		9	114	7.9
6	Chubut	VI	0	72	0
14	Neuquén		1	73	1.4
15	Rio Negro		3	150	2.0
19	Santa Cruz		0	5	0
	Total for area VI		4	300	1.3
	Total for country		142	3 077	4.6

FIG. 4

GEOGRAPHICAL DISTRIBUTION OF SLIDE AND SEROLOGICAL RESULTS BY PUBLIC HEALTH AREAS IN ARGENTINA [a]



Vertical numerals refer to slide rates

Italic numerals refer to percentage serologically positive

[a] Roman numerals (I–VI) refer to public health areas

TABLE 3

ARGENTINA: NUMBER OF SLIDE-POSITIVE MALARIA CASES, 1962–65, FROM ACTIVE AND PASSIVE SURVEILLANCE BY STATE AND PUBLIC HEALTH AREA

State code	Name of state	Public health area	No. positive	No. tested	% positive
4	Corrientes	III	16	12 297	0.13
5	Chaco		4 974	45 375	10.96
8	Formosa		570	14 957	3.81
13	Misiones		246	33 344	0.74
	Total for area III		5 806	105 973	5.5
2	Catamarca	IV	0	17 191	0
9	Jujuy		211	125 938	0.17
16	Salta		441	316 100	0.14
21	Santiago del Estero		11	38 799	0.03
22	Tucumán		21	91 506	0.03
	Total for area IV		687	589 534	0.12
	Total		6 493	695 507	0.93

TABLE 4

ARGENTINA: DISTRIBUTION OF POSITIVE SEROLOGICAL REACTORS TO A *PLASMODIUM KNOWLESI* ANTIGEN FOR MALARIA ANTIBODY BY BLOOD GROUPS

Blood group	Serologically positive		Distribution of sample	
	No.	%[a]	No.	%[a]
A	42	29.6	985	32.0
B	19	13.4	277	9.0
O	78	54.9	1 738	56.5
AB	3	2.1	77	2.5
Total	142	100.0	3 077	100.0

[a] $\chi^2_3 = 3.22$ (P 0.05).

different from the distribution in the samples as a whole.

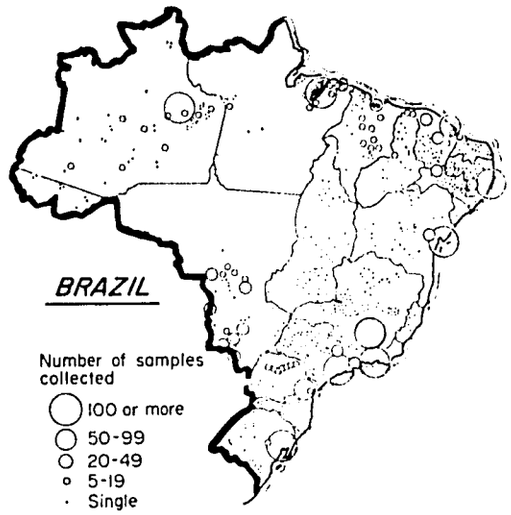
Table 5 shows a comparison of serological and surveillance results for malaria for states from which both data are available. Of the 9 states with high serological prevalence, 6 are also high in the list of states with high slide-positivity rates.

Brazil

The geographical distribution of Brazilian recruits tested is shown in Fig. 5. The largest group of recruits came from states along the eastern seaboard.

FIG. 5

GEOGRAPHICAL DISTRIBUTION OF BRAZILIAN RECRUITS



Few samples were obtained from the central zone, since this area is sparsely populated. Fig. 6 shows the delineation of public health areas and identifies each of the states in Brazil. The number of positive serological reactors by state and public health area is shown in Table 6. The serological prevalence in public health areas varied from 10% to 40%, with an average of 21%.

TABLE 5

ARGENTINA: COMPARISON OF SEROLOGICAL AND SLIDE RESULTS FOR MALARIA

Rank	Serology			Slide examination		
	State code	Name of state[a]	% positive	State code	Name of state[a]	% positive
1	22	Tucumán	28.0	5	Chaco	10.96
2	16	Salta	5.4	8	Formosa	3.81
3	13	Misiones	4.4	13	Misiones	0.74
4	2	Catamarca	2.1	9	Jujuy	0.17
5	9	Jujuy	1.6	16	Salta	0.14
6	4	Corrientes	1.4	4	Corrientes	0.13
7	5	Chaco	0	22	Tucumán	0.03
8	8	Formosa	0	21	Santiago del Estero	0.03
9	21	Santiago del Estero	0	2	Catamarca	0.00

[a] States from which both data were available. States are ranked from highest to lowest prevalence rates.

TABLE 6
BRAZIL: NUMBER OF POSITIVE SEROLOGICAL REACTORS TO A *PLASMODIUM K' OWLESI* ANTIGEN FOR MALARIA ANTIBODY BY STATE AND PUBLIC HEALTH AREA

State code	Name of state	Public health area	No. positive	No. tested	% positive
2	Acre	I	3	8	37.5
3	Roraima		2	2	100.0
4	Amazonas		103	259	39.8
5	Para		94	239	39.3
	Total for area I		202	508	39.8
7	Maranhão	II	35	127	27.6
	Total for area II		35	127	27.6
24	Mato Grosso	III	69	272	25.4
	Total for area III		69	272	25.4
25	Goias	IV	10	39	25.6
26	Distrito Federal		0	5	0
	Total for area IV		10	44	22.7
8	Piaui	V	7	30	23.3
9	Ceará		13	280	4.6
10	Rio Grande do Norte		3	19	15.8
11	Paraiba		3	34	8.8
12	Pernambuco		72	214	33.6
13	Alagôas		1	4	25.0
15	Sergipe		0	8	0
16	Bahia		44	254	17.3
	Total for area V		143	843	17.0
17	Minas Gerais	VI	37	199	18.6
18	Espirito Santo		0	6	0
19	Rio de Janeiro		2	24	8.3
20	São Paulo		16	167	9.6
27	Guanabara		0	45	0
	Total for area VI		55	441	12.5
21	Paraná	VII	9	209	4.3
22	Santa Catarina		3	24	12.5
23	Rio Grande do Sul		32	213	15.0
	Total for area VII		44	446	9.9
	Total		558	2 681	20.8

FIG. 6
STATES AND PUBLIC HEALTH AREAS IN BRAZIL[a]

[a] Roman numerals (I–VII) refer to public health areas, each comprised of 1 or more states. Small numbers refer to individual states listed in Tables 6–8.

The number of slide-positive malaria cases, is determined by active and passive surveillance for the last half of 1963 and the first half of 1964 by state and public health area, is shown in Table 7. Slide-positive rates in the states varied from 0.01% to 16.8%. Public health area IV showed the highest rate of positive slides, 15.7%, whereas only 2% of slides from area V were positive. The over-all slide-positivity rate was 4.7%.

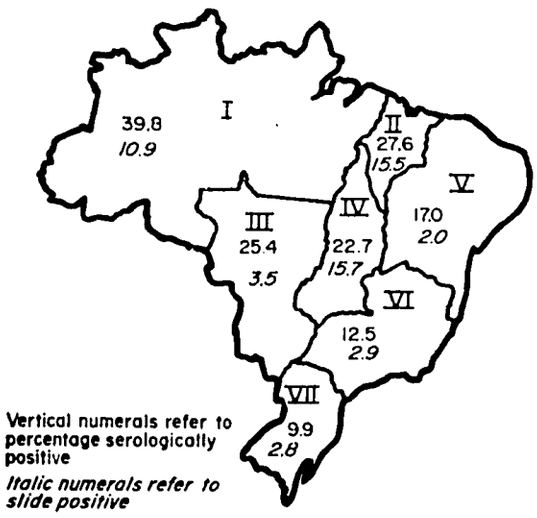
The geographical distribution of slide and serological results is shown in Fig. 7. The serological percentages of positives were higher than the slide positivity percentages and did not vary as much.

Table 8 shows the ranking, from highest to lowest prevalence according to serological and slide examinations, of states for which both types of results were available. Examination of this table shows that 10 of the top 12 states in the "serology" column are also in the top 12 of the "slide examination" column. These results suggest a high degree of correlation.

Colombia

Fig. 8 indic. the geographical distribution of the sampled recruits in Colombia, and Fig. 9 shows

FIG. 7
GEOGRAPHICAL DISTRIBUTION OF SLIDE AND SEROLOGICAL RESULTS BY PUBLIC HEALTH AREAS IN BRAZIL[a]



[a] Roman numerals (I–VII) indicate public health areas.

FIG. 8
GEOGRAPHICAL DISTRIBUTION OF COLOMBIAN ARMY RECRUITS

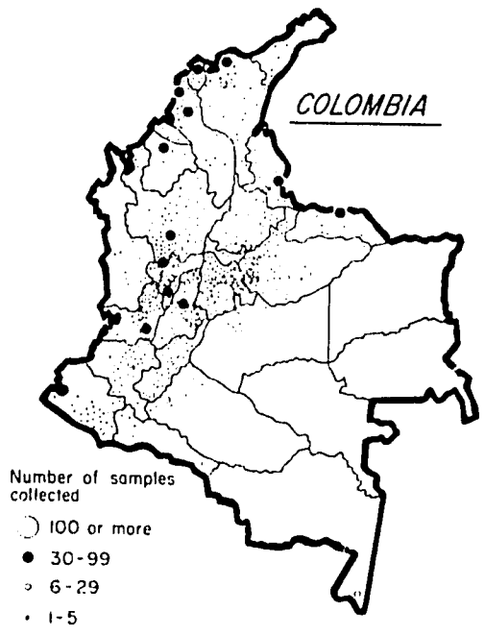


TABLE 7

BRAZIL: NUMBER OF SLIDE-POSITIVE MALARIA CASES, 1963–64, FROM ACTIVE AND PASSIVE SURVEILLANCE BY STATE AND PUBLIC HEALTH AREA

State code	Name of state	Public health area	No. positive	No. tested	% positive
2	Acre	I	130	2 749	4.7
3	Roraima		416	2 480	16.8
4	Amazonas		582	10 624	5.5
5	Para		2 670	18 930	14.1
	Total for area I		3 798	34 783	10.9
7	Maranhão	II	3 825	24 635	15.5
	Total for area II		3 825	24 635	15.5
24	Mato Grosso	III	1 024	28 917	3.5
	Total for area III		1 024	28 917	3.5
25	Goias	IV	4 187	26 589	15.7
26	Distrito Federal		—	—	—
	Total for area IV		4 187	26 589	15.7
8	Piaui	V	878	13 195	6.7
9	Ceará		470	20 236	2.3
10	Rio Grande do Norte		5	58 240	0.01
11	Paraiba		101	27 265	0.37
12	Pernambuco		223	17 336	1.3
13	Alagôas		2	4 808	0.04
15	Sergipe		13	22 114	0.06
16	Bahia		2 131	24 471	8.7
	Total for area V		3 823	187 665	2.0
17	Minas Gerais	VI	1 560	45 757	3.4
18	Espirito Santo		611	13 307	4.6
19	Rio de Janeiro		71	12 664	0.6
20	São Paulo		—	—	—
27	Guanabara		20	6 312	0.3
	Total for area VI		2 262	78 040	2.9
21	Paraná	VII	364	17 828	2.0
22	Santa Catarina		935	28 679	3.3
23	Rio Grande do Sul		—	—	—
	Total for area VII		1 299	46 507	2.8
	Total		20 218	427 136	4.7

TABLE 8

BRAZIL: COMPARISON OF SEROLOGICAL AND SLIDE RESULTS FOR MALARIA

Rank	Serology			Slide examination		
	State code	Name of state [a]	% positive	State code	Name of state [a]	% positive
1	3	Roraima	100	3	Roraima	16.8
2	4	Amazonas	39.8	25	Goias	15.7
3	5	Para	39.3	7	Maranhão	15.5
4	2	Acre	37.5	5	Para	14.1
5	12	Pernambuco	33.6	16	Bahia	8.7
6	7	Maranhão	27.6	8	Piaui	6.6
7	25	Goias	25.6	4	Amazonas	5.5
8	24	Mato Grosso	25.4	2	Acre	4.7
9	13	Alagôas	25.0	18	Espirito Santo	4.6
10	8	Piaui	23.3	24	Mato Grosso	3.5
11	17	Minas Gerais	18.6	17	Minas Gerais	3.4
12	16	Bahia	17.3	22	Santa Catarina	3.3
13	10	Rio Grande do Norte	15.8	9	Ceará	2.3
14	22	Santa Catarina	12.5	21	Paraná	2.0
15	11	Paraiba	8.8	12	Pernambuco	1.3
16	19	Rio de Janeiro	8.3	19	Rio de Janeiro	0.6
17	9	Ceará	4.6	11	Paraiba	0.4
18	21	Paraná	4.3	27	Guanabara	0.3
19	15	Sergipe	0.0	15	Sergipe	0.1
20	18	Espirito Santo	0.0	13	Alagôas	0.0
21	27	Guanabara	0.0	10	Rio Grande do Norte	0.0

[a] States from which both data were available. States are ranked from highest to lowest prevalence rates.

the states and public health areas. Recruits were drawn mainly from the western part of the country. The eastern half of the country (public health areas II and IV) is sparsely populated and consequently contributed few recruits. The data from the whole Colombian survey (Table 9), based on the state of longest residence, indicate that public health areas III and IV, in the interior of the country, had the highest prevalence of positive antibody levels. The central public health area (area II) had the lowest, whereas the coastal areas (areas I and VI showed intermediate antibody levels.

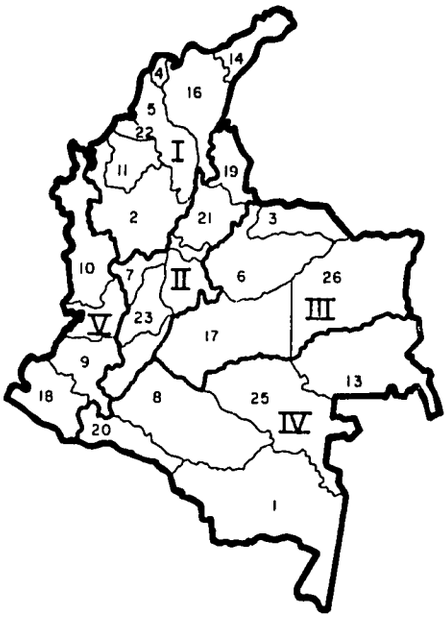
Of the 4 countries included in this study, the recruits from Colombia showed the highest percentage of positive titres to malaria, 21.3%. Note (Fig. 1) that the peak titre of the Colombian recruits was 1 : 16, whereas the peak titre of recruits from Argentina, Brazil, and the USA was 1 : 8. This may indicate more intense exposure to malaria than is found in the other countries studied.

The number of slide-proven malaria cases for the years 1963, 1964, and 1965 obtained by active and passive surveillance, is shown in Table 10. The overall prevalence by slide examination was 3%. The geographical distribution of slide and serological results by public health area is shown in Fig. 10.

Table 11 shows the ranking, from highest to lowest prevalence, according to serology and slide examination. The table shows that 8 of the top 10 states in the "serology" column rank in the

FIG. 9

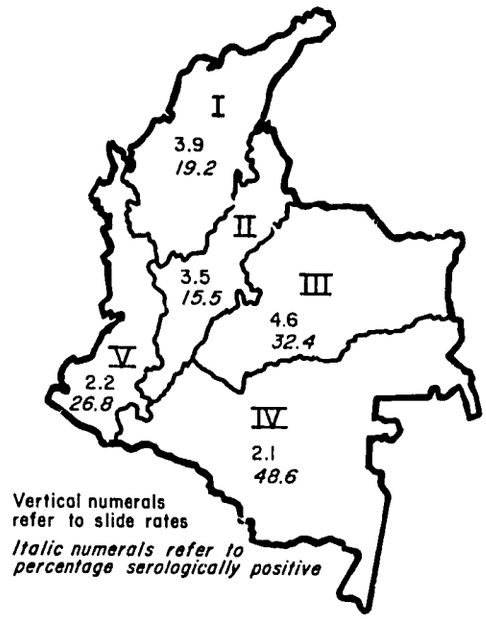
STATES AND PUBLIC HEALTH AREAS IN COLOMBIA [a]



[a] Roman numerals (I–V) refer to public health areas, each comprised of 1 or more states. Small numbers refer to individual states listed in Tables 9–11.

FIG. 10

GEOGRAPHICAL DISTRIBUTION OF SLIDE AND SEROLOGICAL RESULTS BY PUBLIC HEALTH AREAS IN COLOMBIA [a]



[a] Roman numerals (I–V) indicate public health areas.

first 10 states in the "slide examination" column. These data again support the results obtained by serological testing in the country.

Table 12 shows that the incidence of malaria antibody is related to altitude, as one would expect. Malaria, as evidenced by antibody prevalence, has the highest prevalence in altitudes between sea level and 200 m. Antibody in the recruit population declined sharply at higher altitudes and levelled off above 1500 m. Malaria transmission is believed to occur up to the 1500 m–2000 m levels. This altitude factor may explain the relatively low antibody activity among persons from public health area II. This region, which lies along the Andes chain, is largely mountainous.

No specific correlations were found between blood types in the ABO system and the presence of malaria antibody (Table 13).

The data from lifelong residents of the same area follow the same patterns as data from the entire sample which is based on the state of longest residence. The over-all percentage of positives is somewhat, but not significantly, lower. The lower percentage may be due to a smaller number of specimens being available in this category.

United States of America

The geographical distribution of the 30 serological reactors to the malaria antigen in the USA, by state of birth, gave no indication of a pattern. Individuals whose sera had reacted in the test lived from Maine to California. This suggests that the reactions observed were probably non-specific. The fact that almost all of the sera were of negative titre also attests to the test's high degree of specificity and non-reactivity.

Of the 2237 USA sera tested, 34 (1.5%) reacted to malaria antigen, but only 20 reacted in serum dilutions of 1 : 8 or greater. Of the positive reactors, 12 out of 20 (60%) were from a rural environment, 6 out of 20 (30%) were from towns, and 2 out of 20 (10%) were from urban areas.

TABLE 9

COLOMBIA: THE NUMBER OF POSITIVE SEROLOGICAL REACTORS TO A *PLASMODIUM KNOWLESI* ANTIGEN FOR MALARIA ANTIBODY BY STATE AND PUBLIC HEALTH AREA

State code	Name of state	Public health area	State of longest residence			Birthplace		
			No. positive	No. tested	%	No. positive	No. tested	%
2	Antioquia	I	43	200	21.5	16	91	17.6
4	Atlantico		4	43	9.3	3	24	12.5
5	Bolivar		32	145	22.0	23	92	25.0
11	Córdoba		6	21	28.6	2	10	20.0
14	Guajira		1	8	12.5	1	1	100.0
16	Magdalena		23	150	15.3	4	81	4.9
Total for area I			109	567	19.2	49	299	16.4
7	Caldas	II	47	364	12.9	25	205	12.2
12	Cundinamarca		45	404	11.1	19	207	9.2
15	Huila		13	100	13.0	1	48	2.1
19	Norte de Santander		33	105	31.4	24	74	32.4
21	Santander		49	179	27.4	24	95	25.3
23	Tolima		35	281	12.5	19	149	12.8
Total for area II			222	1 433	15.5	112	778	14.4
3	Arauca	III	30	45	66.7	4	8	50.0
6	Boyaca		101	368	27.4	40	174	23.0
17	Meta		10	22	45.5	1	4	25.0
Total for area III			141	435	32.4	45	186	24.2
1	Amazonas	IV	2	7	28.6	0	1	0
8	Caqueta		12	34	35.3	2	14	14.3
20	Putumayo		22	33	66.7	5	9	55.6
Total for area IV			36	74	48.6	7	24	29.2
9	Cauca	V	24	124	19.4	14	79	17.7
10	Choco		45	55	81.8	28	33	84.8
18	Narino		11	63	17.5	5	28	17.9
24	Valle (del Cauca)		42	213	19.7	15	87	17.2
Total for area V			122	455	26.8	62	227	27.3
Total sample			630	2 964	21.3	275	1 514	18.2

TABLE 10

COLOMBIA: NUMBERS OF SLIDE-PROVEN MALARIA CASES (1963, 1964, 1965) BY STATE AND PUBLIC HEALTH AREA

State code	Name of state	Public health area	No. positive	No. tested	% positive
2	Antioquia		10 658	210 055	5.1
4	Atlantico		182	27 587	0.6
5	Bolivar		1 215	91 289	1.3
11	Córdoba		9 608	119 617	8.0
14	Guajira		204	26 245	0.8
16	Magdalena		657	99 738	0.6
Total for area I			22 524	574 531	3.9
7	Caldas		588	61 040	1.0
12	Cundinamarca		1 804	91 269	2.0
15	Huila	II	52	34 059	0.2
19	Norte de Santander		3 996	61 432	6.5
21	Santander		6 551	73 921	8.9
23	Tolima		1 030	81 982	1.2
Total for area II			14 021	403 703	3.5
3	Arauca		980	22 371	4.4
6	Boyaca	III	2 930	84 814	3.4
17	Meta		2 383	30 824	7.7
Total for area III			6 293	138 009	4.6
1	Amazonas		0	348	0
8	Caqueta	IV	164	32 171	0.5
20	Putumayo		757	11 976	6.3
Total for area IV			921	44 495	2.1
9	Cauca		2 260	72 077	3.1
10	Choco	V	1 790	57 886	3.1
18	Narino		2 478	108 756	2.3
24	Valle (del Cauca)		774	85 184	0.9
Total for area V			7 302	323 903	2.2
Total sample			51 061	1 484 641	3.4

TABLE 11

COLOMBIA: COMPARISON OF SEROLOGICAL AND SLIDE RESULTS FOR MALARIA

Rank	Serology						Slide examination		
	State of longest residence			Birthplace					
	State code	Name of state [a]	% positive	State code	Name of state [a]	% positive	State code	Name of state [a]	% positive
1	10	Choco	81.8	14	Guajira	100.0	21	Santander	8.9
2	3	Arauca	66.7	10	Choco	84.4	11	Córdoba	8.0
3	20	Putumayo	66.7	20	Putumayo	55.6	17	Meta	7.7
4	17	Meta	45.4	3	Arauca	50.0	19	Norte de Santander	6.5
5	8	Caqueta	35.2	19	Norte de Santander	32.4	20	Putumayo	6.3
6	19	Norte de Santander	31.4	21	Santander	25.3	2	Antioquia	5.1
7	1	Amazonas	28.6	17	Meta	25.0	3	Arauca	4.4
8	11	Córdoba	28.6	5	Bolivar	25.0	6	Boyaca	3.4
9	6	Boyaca	27.8	6	Boyaca	23.0	9	Cauca	3.1
10	21	Santander	27.4	11	Córdoba	20.0	10	Choco	3.1
11	5	Bolivar	22.0	18	Narino	17.8	18	Narino	2.3
12	2	Antioquia	21.5	9	Cauca	17.7	12	Cundinamarca	2.0
13	24	Valle (del Cauca)	19.7	2	Antioquia	17.6	5	Bolivar	1.3
14	9	Cauca	19.4	24	Valle (del Cauca)	17.2	23	Tolima	1.2
15	18	Narino	17.5	8	Caqueta	14.3	7	Caldas	1.0
16	16	Magdalena	15.3	23	Tolima	12.8	24	Valle (del Cauca)	0.9
17	15	Huila	13.0	4	Atlantico	12.5	14	Guajira	0.8
18	7	Caldas	12.9	7	Caldas	12.2	4	Atlantico	0.6
19	14	Guajira	12.5	12	Cundinamarca	9.2	16	Magdalena	0.6
20	23	Tolima	12.5	16	Magdalena	4.9	8	Caqueta	0.5
21	12	Cundinamarca	11.1	15	Huila	2.1	15	Huila	0.2
22	4	Atlantico	9.3	1	Amazonas	0.0	1	Amazonas	0.0

[a] States from which both data were available. States are ranked from highest to lowest prevalence rates.

TABLE 12

COLOMBIA: DISTRIBUTION OF POSITIVE SEROLOGICAL REACTORS BY ALTITUDE

Altitude (m)	No. positive	No. examined	% positive	Cumulative percentage of total no. positive
0–200	174	516	33.7	28.3
200–500	112	404	27.7	46.6
500–1000	49	254	19.3	54.6
1000–1500	105	527	19.9	71.7
1500–2000	85	590	14.4	85.5
2000–3000	84	575	14.6	99.2
3000–4000	5	32	15.6	100.0
Total	614	2 898	21.2	

TABLE 13

DISTRIBUTION OF POSITIVE SEROLOGICAL REACTORS BY BLOOD GROUPS FOR COLOMBIA

Blood group	Serologically positive		Distribution of sample	
	No.	%	No.	%
A	152	24.1	711	25.1
B	62	9.8	250	8.8
O	405	64.3	1 905	64.3
AB	11	1.7	55	1.9
Total	630		2 951	

[a] $\chi^2_{(3)} = 0.89$ ($P > 0.05$).

DISCUSSION

The indirect haemagglutination test using *P. knowlesi* antigen has been shown to be a sensitive and reproducible method for demonstrating antibody to human malaria (Rogers, Fried & Kagan, 1968). Desowitz & Saave (1965) used an IHA test employing formolized tanned sheep cells sensitized with *P. cynomolgi* and *P. coatneyi* antigens to study areas of different malaria endemicity. Their results showed reduced antibody levels in protected populations over the antibody levels in populations exposed to malaria.

The serological data obtained in this study could not be related directly to the malarial history of the donor. For this reason an epidemiological approach was made to the evaluation of the data. Except for Argentina, serological data on the distribution of malaria in the 4 countries sampled compare well with data obtained by active surveillance. One would normally expect the seropositivity rate to exceed the slide-positivity rate several-fold, since the former may be measuring lifelong experience with the parasite, whereas the latter measures active infection at the moment of examination. In the states of Chaco, Formosa, and Santiago del Estero in Argentina, the slide-positivity rates were higher than the seropositivity rates. These differences may be due, in part, to differences in sampling, since recruits are of restricted ages, and the people in the surveillance surveys are of all ages. The extremely large difference between the serologically positive samples (28%) and the slide-positive (0.02%) figures in the state of Tucumán is puzzling. These data suggest either that the microscopical techniques were inadequate in this area or that the seropositivity index is erroneous. The lack of reactivity in the serological tests with the USA sample suggests that the test used did not yield a high rate of false positivity. A comparison of data obtained by serology and by active and passive surveillance in Brazil and Colombia strongly suggests that both methods are measuring prevalence of malaria, since with a few exceptions, states with the highest slide-positive rates also ranked high in serological reactivity. Discrepancy in the 2 sets of data for Tucumán is probably due to the fact that the IHA test for malaria detects antibody from experience with malaria as far back as 5 years or more. Active surveillance detects only the cases of malaria present at the time of sampling. One would logically expect much higher levels by the serological method. Years ago, Tucumán was an area of high endemicity, and this may be reflected in the serological data.

The over-all distribution of serological reactors in Argentina obtained in this study is plausible. Bejarano (1962) reviewed the epidemiology of malaria in Argentina for the years 1897–1960, and recorded 9 cases of malaria in the State of Mendoza in 1959 (the last year reviewed). In our study we found a seropositivity rate of 10.7% among the 84 samples from Mendoza. In San Luis, 5% of the 60 samples were seropositive. Malaria was common in this state in the 1940s and 1950s. The last case was reported in 1958. The geographical distribution in Argentina of the 3 anopheline vectors, *Anopheles pseudopunctipennis*, *A. albitarsis*, and *A. darlingi*, coincides very closely with the serological range of reactors obtained in this study (Bejarano, 1959). Malaria may not exist in these areas today, but it did exist in the not too distant past, and the serological method may be detecting this event.

Athreya & Coriell (1967) discussed the significance of high B-gene frequency in population groups in various parts of the world in relation to endemic malaria. They postulated that the presence of blood group-A substance in malaria may be responsible for increased pathogenicity and mortality in individuals in group A thus causing a shift in the gene frequency in the population. Raper (1968) analysed data from 3500 patients experimentally infected with malaria in the treatment of neurosyphilis and other disorders. Raper could not find any correlation between parasitaemia and the presence of anti-A or anti-B in the blood of the patients. Our inability to find any correlation between serological reactivity and the ABO status of the 6038 young men sampled in Argentina and Colombia confirms this finding.

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RÉSUMÉ

ÉTUDES SÉRO-ÉPIDÉMIOLOGIQUES DU PALUDISME PAR L'ÉPREUVE D'HÉMAGGLUTINATION INDIRECTE: PRÉLÈVEMENTS DE SÉRUMS CHEZ DES RECRUES D'ARGENTINE, DU BRÉSIL, DE COLOMBIE ET DES ÉTATS-UNIS D'AMÉRIQUE

On a eu recours à l'épreuve d'hémagglutination indirecte, utilisant comme antigène *Plasmodium knowlesi* adsorbé sur des érythrocytes humains du groupe O, pour titrer les anticorps antipaludéens dans 10 956 sérums prélevés sur des recrues de quatre pays de l'hémisphère occidental. Un titre de 1/8 ou plus a été choisi comme seuil de positivité dans cette enquête épidémiologique. Sur la base de ce critère, la prévalence des réactions sérologiques positives a été la suivante: États-Unis d'Amérique: 20 sérums positifs sur 2237 (0,9%); Argentine: 142 sur 3077 (4,6%); Brésil: 558 sur 2681 (20,8%); Colombie: 629 sur 2961 (21,3%).

Aux États-Unis, l'analyse de la provenance des sérums positifs a montré la diversité de leur origine et l'absence de tout foyer, ce qui donne à penser qu'il pourrait s'agir de réactions non spécifiques. En Argentine, on a décelé la présence d'anticorps chez des recrues dans des régions où aucun cas de paludisme n'avait été signalé depuis 10 ans au moins; il semble que l'épreuve d'hémagglutination mette en évidence des anticorps résiduels dans une population plusieurs années après l'éradication du paludisme. Au Brésil, on a relevé une corrélation satisfaisante entre les résultats des examens sérologiques et la répartition du paludisme déterminée par les examens parasitologiques directs. C'est en Colombie que les taux de positivité ont été les plus élevés, les titres de 1/16 étant les plus nombreux alors que dans les trois autres pays le titre de 1/8 était le plus fréquemment observé. Dans ce pays également, on a noté une concordance satisfaisante entre la prévalence du paludisme établie par l'enquête sérologique et la prévalence attestée par les résultats des examens parasitologiques. Plus de 50% des sérums positifs provenaient de recrues colombiennes stationnées dans des camps situés à moins de 1000 m d'altitude.

En Argentine et en Colombie, aucune corrélation n'est apparue entre la présence des anticorps antipaludéens et l'appartenance à l'un ou l'autre groupe sanguin du système ABO.

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