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FURTHER STUDIES OF THE XAVANTE INDIANS

IX. IMMUNOLOGIC STATUS WITH RESPECT TO VARIOUS DISEASES AND ORGANISMS

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Previous papers in this series have attempted to describe in some detail the Xavante Indians of the Brazilian state of Mato Grosso and to begin to define some of the biological pressures to which they are subject.[1, 4] In this communication, we will describe the results of tests of immunologic status with respect to various diseases and organisms. The Indians are from two villages, one near the Simões Lopes Post of the Indian Protective Service and the other near the Salesian Mission of São Marcos, both in Mato Grosso. Quite aside from the linguistic problem, Indian interests and attitudes toward disease make a precise medical history extremely difficult to obtain. Accordingly, the extensive study of antibody profiles offers perhaps the best available means of ascertaining the agents of disease to which these Indians have been exposed.

The primary, over-all objective of these studies is to contribute to an understanding of the population genetics of primitive man, the term *population genetics* being used in a wide sense, including, for instance, the mortality structure and the agents responsible for it. The antibody and related studies can in this context fulfill two functions. They can, on the one hand, indicate the extent to which the "diseases of civilization" (measles, pertussis, tuberculosis, etc.) have reached this group and may have produced departures from the "pre-contact" picture. They can, on the other hand, help to identify endemic-disease pressures to which the Indian may long have been accustomed. Although there has been a certain opportunism in the choice of the tests, in general an effort was made to select those tests that would reflect exposure to certain major categories of disease-producing fungi, viruses, bacteria, and parasites. Since the spectrum that might be investigated includes several hundred kinds of antibodies, it will be apparent that only a start has been made on a full definition of the agents of disease to which the Xavante are subject. Even so, a number of unexpected findings emerge already.

All available members of the two villages were tested. Blood specimens were drawn into vacuum containers with ethylenediaminetetra-acetic acid (EDTA) as anticoagulant; it was not often practical to bleed children under the age of 2 years. All findings were tabulated and analyzed on the basis of the three age groupings employed in other papers in the series, namely, 0–14, 15–30, and 31 years or greater. In the interest of conserving space these tabulations will not be presented, but where there is a significant age effect, this will be stated. A finer breakdown by age would of course be desirable, but is not justified at this time by the numbers involved. Since these Indians live in small villages well dispersed over large areas, the amassing of large numbers of persons is much more difficult than in Tropical agricultural populations.

INTRADERMAL TESTING WITH TUBERCULIN, COCCIDIOIMYCIN, AND HISTOPLASMIN

Coccidioidin, histoplasmin, and tuberculin were injected intradermally in the flexor surface of the forearm in succession: coccidioidin near the antecubital fossa, histoplasmin in the mid-portion, and tuberculin in the most distal portion of the forearm. The results were judged about 72 hours after injection. Regardless of the extent of the erythema, a reaction was considered positive

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TABLE 1

Results of skin tests with coccidioidin, histoplasmin, and tuberculin at Simões Lopes

Sex	Coccidioidin			Histoplasmin			Tuberculin		
	Examined (*No.*)	Positive		Examined (*No.*)	Positive		Examined (*No.*)	Positive	
		(*No.*)	(%)		(*No.*)	(%)		(*No.*)	(%)
M	47	1	2.1	45	25	55.6	47	0	0
F	37	1	2.7	37	10	27.0	37	1	2.7
M & F	84	2	2.4	82	35	42.7	84	1	1.2

only if there were induration at the site of injection. The tuberculin was Parke, Davis & Co. PPD, injected in an "intermediate" dose of 0.0001 mg in 0.1 cc of buffered diluent. The coccidioidin was obtained from Cutter Laboratories; 0.1 cc of a 1:100 material was injected. The histoplasmin was obtained from Parke, Davis & Co.; 0.1 cc of a solution prepared as recommended was used. All solutions were prepared just before use. Nine persons were injected with 0.1 cc of sterile saline solution as a control on cutaneous hyper-reactivity—in none did erythema or induration occur at the injection site.

The results are given in Table 1. There were only two positive responses to coccidioidin and one to tuberculin. One 51-year-old woman (recorded as histoplasmin-negative) contributed two of these three positive responses (one of the two coccidioidins and the tuberculin). In view of the improbability of this finding, we are inclined to attribute it to a confusion of the test antigens. Whether this is the explanation of the remaining positive response to coccidioidin is uncertain. That there were no other responses to tuberculin in this group is of course confirmatory evidence of the paucity of their contacts with neo-Brazilians and other "outsiders." But, on the other hand, it is clear that this is a region of endemic histoplasmosis, 42.7 ± 5.5% of the persons tested responding positively. In 31 of the 35 positive reactions, the area of induration was 10 mm or more in greatest diameter. Males reacted significantly more often than females ($\chi^2 = 6.76$, d.f. = 1, $0.01 > P > 0.001$) and adults (15 years and older) more than children (children, three of 26 positive; adults, 32 of 56 positive, $\chi^2 = 15.10$, d.f. = 1, $P < 0.001$). This age effect of course suggests the gradual acquisition of sensitivity throughout a considerable part of life, in contrast to the findings regarding *Salmonella* and poliovirus (see below), the antibodies to which are acquired during childhood.

TABLE 2

Titer of antibodies to Toxoplasma *and* Plasmodium *among Xavante Indians from São Marcos*

Sex	*Toxoplasma*				*Plasmodium*			
	Titer			Total	Titer			Total
	≤108	324–972	≥2,916		0–5	15–45	≥135	
M	0	0	42	42	15	25	4	44
F	1	3	61	65	27	31	7	65
M & F	1	3	103	107	42	56	11	109

ANTIBODIES TO *TOXOPLASMA*

Physical examination at Simões Lopes of an adult Xavante with the type of inactive chorioretinitis often associated with congenital toxoplasmosis[5] prompted a systematic search for serologic evidences of infection with this organism. Antibodies were identified by the indirect hemagglutination test for toxoplasmosis.[6] Threefold serial dilutions of sera were prepared, beginning at a dilution of 1:12. Dilutions were not carried beyond 1:78,732. Endpoints were expressed as the reciprocal of the final dilution giving agglutination of 2+ or greater (Table 2). Of 107 sera obtained in São Marcos, all were positive. Reactivity is indicated as low (titer 108 or below), moderate (324 to 972), and high (greater than 972). Known positive and negative sera were tested simultaneously as controls. Chordi, Walls, and Kagan[7] have indicated that hemagglutination titers below 1:200 may represent nonspecific reactivity, whereas titers in excess of 1:200 have been specific. It should be noted that only one of 107 sera fell in the low-

reactivity group, and three in the moderate group.

With such a high frequency of positive reactions, there is little scope for a sex difference ($\chi^2 = 2.685$, d.f. = 2, $.30 > p > .20$) but it may be significant that the lower titers just mentioned were in females. The sample included only three persons under the age of 15 years; the one low titer encountered was in a child. Twenty of the sera were further tested by the methylene-blue dye test. Nineteen of these were high titer in the hemagglutination test. Five of these gave dye-test titers of 512, and the remainder titers of 1,024, the highest dilution measured. The remaining serum had a hemagglutination titer of 36 and was negative by dye titer at a dilution of 1:10. The previously reported correspondence between the results of the hemagglutination and dye tests[7] is thus confirmed. The serologic evidence suggests that this region of the Mato Grosso represents a focus of toxoplasmosis of high endemicity. This is, in fact, the highest prevalence of positive serologic reactivity yet recorded, exceeding the prevalence encountered in such other Tropical areas as Costa Rica (85%),[8] Guatemala (84%),[8] Tahiti (70%),[9] and Honduras (81%).[9]

ANTIBODIES TO PLASMODIA

Whether malaria is a disease of long-standing in the New World or a post-Columbian introduction, and its distribution prior to the last 100 years, are controversial.[10] There are relatively few systematic observations on its prevalence in Indian populations as remote as the Xavante. The blood specimens for antibody studies were obtained at the height of the dry season, when malaria transmission is at its lowest. Although blood films were not obtained nor physical examinations performed in the village in which the serum samples were obtained (São Marcos), in another similar Xavante village in the Mato Grosso studied just 1 week previously (Simões Lopes), only one among 153 blood films was positive for malaria (*Plasmodium vivax*). Only five among 209 persons had slight to moderate splenomegaly.[5]

Malaria antibody was determined by a modification of the indirect fluorescent-antibody test (IFA).[11] *Plasmodium cynomolgi* was used as antigen following the suggestion of Kuvin and Voller.[12] Plasma was prepared in threefold dilutions, beginning at 1:5. The titer represents the reciprocal of the final dilution of plasma that will give a fluorescence with the *P. cynomolgi* test antigen of 2+ or greater when reacted in the IFA test. The antibody measured is group-specific for plasmodia and not specific for species of malaria parasite (Table 2). Of 109 specimens of plasma tested (with two additions, the same as tested for *Toxoplasma* antibodies), 67 were positive, 56 had a moderate titer (15–45) and 11 had high titer (135 or greater). Titers of less than 15 are not considered positive because antibody-free control sera can give titers in this range. There is no difference between the sexes ($\chi^2 = 0.876$, d.f. = 2, $.70 > P > .50$), nor any age effect, but few children were included in this sample. There are few studies of serologic reactivity by the IFA test of populations in areas of endemic malaria and none from South America. However, on the basis of other studies with the IFA test,[12-15] these results suggest that the malaria pressure on this group, while not insignificant, is not severe.

ANTIBODIES TO *TREPONEMA*

The argument over the origin of syphilis—New World or Old—is even brisker than that over the origin of the malaria now present throughout the New World. Twenty-two plasma specimens from São Marcos were subjected to three different tests for anti-treponemal activity. These tests were: 1) the Venereal Disease Research Laboratory (VDRL) slide qualitative flocculation test,[16] 2) the Kolmer one-fifth volume (KRP) test,[16] and 3) the fluorescent treponemal antibody absorbed (FTA-Abs) test.[17, 18]

Because of the limited plasma available, it was impossible to perform all three tests on every blood specimen. Accordingly, VDRL tests were run on 19 specimens—all were non-reactive. KRP tests were run on 12 specimens—one of these was weakly reactive. Finally, 19 specimens were subjected to the FTA-Abs test—all were non-reactive. The single plasma found weakly reactive with the KRP test was non-reactive to the VDRL and FTA-Abs tests, suggesting that the KRP test result is a false positive. Previous studies for treponemal antibodies in a different village of this same tribe also failed to reveal any unequivocal positive reactions.[1] There is thus no serologic evidence for the occurrence of treponemal infections in this tribe; this finding is supported by our failure to observe clinical signs of syphilis, yaws, or pinta.

ANTIBODIES TO *SALMONELLA* ANTIGENS

Evidence of the presence of antibodies to the *Salmonella* group of enteric bacterial pathogens was sought with the indirect fluorescent-antibody (FA) technique. Cultures of *Salmonella paratyphi* A (Group A), *S. typhimurium* (Group B), *S. thompson* (Group C-1) and *S. panama* (Group D) were grown in trypticase soy agar slants, suspended in buffered saline solution, and diluted to a density of the MacFarlane #1 standard and used to prepare smears on slides. The slides were gently heat-fixed. Plasma was diluted 1 to 10 in buffered saline solution, inactivated at 56°C for 30 minutes, and then centrifuged to remove any sediment. With a Wright pipette, plasma was added to cover the smear, allowed to react for 30 minutes at room temperature, and then the slide was washed in buffered saline solution for 10 minutes. The slides were then well drained, and a 1 to 8 dilution of a fluorescein isothiocyanate-labeled anti-human gamma globulin solution was added and allowed to act for 30 minutes. Next the slides were again washed in buffered saline solution for 10 minutes and then mounted, buffered glycerol (pH 7.8), being used.

The slides were examined under a Zeiss GFL microscope, with an Osram HBO 200 high-intensity light source. The filters used included a BG 12 exciter filter with a combination of OG 4 and GG4 as barrier filters. Readings were made on a graduated basis, a 4+ recording brilliant fluorescence of the organisms, while 3+ and 2+ denoted decreasing brilliance of fluorescence (Table 3). Reactions below this level (1+ and ±) were not scored as positive, since these weak reactions have been shown, in other studies, not to be significant.

The results of a χ^2 analysis, employing the arc–sine transformation, of the data of Table 3, combined with the previously published data from the Xavante village near São Domingos,[1] are given in Table 4. For these analyses the data have also been partitioned into the three standard age groups, 40.7% of the total sample of 297 being less than 15 years of age. The generally high frequency of positive responders to the four antigens, which was previously observed in the São Domingos study, is confirmed by the

TABLE 3

Frequency of occurrence of antibodies to the Salmonella *antigens in two Xavante villages**

Salmonella type	Sex of those tested	Village					
		Simões Lopes			São Marcos		
		Total	Positive		Total	Positive	
			(*No.*)	(%)		(*No.*)	(%)
A	M	71	15	21.1	52	14	26.9
	F	54	19	35.2	57	19	33.3
	M & F	125	34	27.2	109	33	30.3
B	M	71	37	52.1	52	35	67.3
	F	54	35	64.8	57	42	73.7
	M & F	125	72	57.6	109	77	70.6
C	M	71	52	73.2	52	26	50.0
	F	54	46	85.2	57	34	59.6
	M & F	125	98	78.4	109	60	55.0
D	M	71	21	29.6	52	20	38.5
	F	54	20	37.0	57	22	38.6
	M & F	125	41	32.8	109	42	38.5

* Specimens of serum, tested with the fluorescent-antibody technique, yielding a 2+, 3+, or 4+ reaction were scored as positive.

TABLE 4

Results of a χ^2 analysis, employing the arc-sine transformation, of percentage of positive reactions by age, sex, and village, to four different Salmonella *antigens**

Source	*Salmonella* A			*Salmonella* B		
	χ^2	df	P	χ^2	df	P
Village	4.711	2	.10–.05	5.247	2	.10–.05
Sex	2.370	1	.20–.10	0.760	1	.50–.30
Age	0.574	2	.80–.70	6.166	2	.05–.02
Interaction	18.839	12	.10–.05	20.931	12	.10–.05
Total	26.494	17	.10–.05	33.104	17	.02–.01
Source	*Salmonella* C			*Salmonella* D		
	χ^2	df	P	χ^2	df	P
Village	23.790	2	<.001	16.897	2	<.001
Sex	1.997	1	.20–.10	1.964	1	.20–.10
Age	1.576	2	.50–.30	2.671	2	.30–.20
Interaction	12.931	12	.50–.30	11.784	12	.50–.30
Total	40.294	17	.01–.001	33.316	17	.02–.01

* The analysis is based on results previously presented for São Domingos[4] as well as on the findings of Table 4.

present findings, with values roughly from 27% positives to *Salmonella* type A to 78% to *Salmonella* type C. The χ^2 analysis reveals significant total values for three of the four antigens. Sex and age differences are of little moment, the significant total values being primarily due to village differences. For the two antigens (C and D) for which village differences are clearly significant, the effect is due to a relatively low frequency of positives for C in São Marcos and a relatively high frequency for D at São Domingos. The implication is that even with the relative environmental uniformity of Xavante life, and the intervillage migration, the importance of specific *Salmonella* strains varies from village to village. Finally, a separate χ^2 analysis with the arc–sine transformation of the data of Table 3 reveals that the differences within villages in the frequency of responders to the four *Salmonella* antigens are highly significant, A and D positives being less common than B and C positives. In this analysis a significant sex difference emerges, females in general having demonstrable antibodies more often than males.

In view of the newness of the FA procedure, it is not yet possible to evaluate fully the results obtained in this study with respect to the Salmonelleae. About all that can be said with assurance is that antibodies to the *Salmonella* somatic fractions 1, 2, 4, 5, 6, 7, 9, and 12 are present in many of these sera, with village differences as noted. Whether the exposure to certain strains of *Salmonella* that is implied occurred in the course of outbreaks of disease or of constant exposure to small numbers of these organisms over a period of time is unknown.

ANTIBODIES TO *BORDETELLA PERTUSSIS*

The presence of antibodies to the pertussis organism was determined by essentially the same indirect fluorescent-antibody (FA) technique as in the study on *Salmonella* antibodies, except that conversion of the serum to plasma was accomplished by coagulation at 56°C for 30 minutes followed by centrifugation after the specimen had stood overnight at 4°C. The antigen employed to prepare the slide was a standard *Bordetella pertussis* antigen diluted to 20 billion per ml. The results are shown in Table 5. The results of a χ^2 analysis, employing the arc–sine transformation, of the data of Table 5 combined with the previously published data from São Domingos, and including age as a separate parameter (since 40.9% of the total sample of 296 was less than 15 years of age), are shown in Table 6. There is a uniformly high

frequency of positive responses, (about) 60%, which is not related to village, age, or sex.

The indirect FA procedure is currently employed rather extensively in the detection of antibodies to pertussis in whole sera and various fractions of it. In infants recently immunized with pertussis vaccine, the appearance of positive FA tests corresponds to the development of positive agglutination tests.[19] In the present study, 27 serum specimens from persons with positive FA tests were examined with agglutination procedures; all were found to have significant titers. In carefully studied populations, the acquisition of antibodies to pertussis corresponds to outbreaks of the disease. While we cannot be certain this is also the case in the Xavante, it does seem probable all three villages have seen major outbreaks of pertussis; one has been documented in Simões Lopes.[3]

TABLE 5

Frequency of occurrence of antibodies to Bordetella pertussis *in two Xavante villages**

Sex	Village					
	Simões Lopes			São Marcos		
	Total	Positive (*No.*)	(%)	Total	Positive (*No.*)	(%)
M	71	37	52.1	52	35	67.3
F	54	36	66.7	57	28	49.1
M & F	125	73	58.4	109	63	57.8

* The fluorescent-antibody technique was employed; only specimens of serum yielding a 2+, 3+, or 4+ reaction were scored as positive.

TABLE 6

Results of a χ^2 *analysis, employing the arc-sine transformation, of percentage of positive reactions by age, sex, and village, for* Bordetella *pertussis antigen**

Source	χ^2	df	P
Village	1.944	2	.50–.30
Sex	0.038	1	.90–.80
Age	1.266	2	.70–.50
Interaction	27.684	12	.01–.001
Total	30.932	17	.05–.02

* The analysis is based on results from São Domingos[1] as well as the two villages for which results are given in Table 6.

ANTIBODIES ASSOCIATED WITH STREPTOCOCCAL INFECTIONS

The sera of 104 Xavante Indians from the village near São Marcos were assayed for antibody titers to three extracellular antigens of group A streptococci. In general, the specimens were aliquots of the same samples analyzed for antibodies to plasmodia and *Toxoplasma;* as mentioned when these studies were presented, only three of the persons were below the age of 15. Anti-streptolysin O (ASO), anti-diphosphopyridine nucleotidase (anti-DPNase or anti-NADase), and anti-DNase B were selected as three antibodies that most frequently reflect the incidence and intensity of group A streptococcal infections.[20, 22]

The ASO titer was determined by the method of Rantz and Randall,[23] with minor modifications. After the preparation and partial purification of the streptolysin O, its unitage was standardized against standard antisera originally established by Todd.[24] All plasma were assayed with the same lots of streptolysin O. At least two standard sera of known titer were assayed with each run of unknown plasma. In our hands, average ASO titers are usually less than 50 units per ml in infants and less than 200 units per ml in adults who are free of recent streptococcal disease. Titers in school children vary by season and geographic location but average between 200 and 300 units per ml during the winter and spring months in large American cities of the North Temperate Zone (Chicago, New York, Boston, Cleveland, etc.).

The anti-DPNase (NADase) titration was made by a modification of the method described by Bernhard and Stollerman.[21] Streptococcal DPNase was prepared from the same strain of group A streptococci used to prepare streptolysin 0 (C203S) and was concentrated in the same purified fraction of the broth supernatant that contained streptolysin 0 activity as described by Bernheimer, Lazarides, and Wilson.[25] A reference standard antiserum was employed that was originally standardized by Dr. Alan W. Bernheimer, but the unitage was modified for closer comparability with the ASO titer. Thus, a large pool of normal adult serum containing 125 units per ml of antistreptolysin 0 assayed 80 units per ml of anti-DPNase. Anti-DPNase titers greater

TABLE 7

Titers of antistreptolysin O, anti-DNase-B, and anti-NADase among Xavante Indians from the village at São Marcos

A. Antistreptolysin O

Sex	Titer					Total
	<50	62–83	100–166	200–250	>300	
M	8	19	10	2	0	39
F	4	30	14	14	1	63
M & F	12	49	24	16	1	102
	$\chi^2 = 9.277^*$ d.f. = 3 p .05–.02					

B. Anti-DNAse-B

Sex	Titer					Total
	<20	20–60	80–160	240–480	640–1280	
M	2	4	16	14	4	40
F	10	7	34	6	7	64
M & F	12	11	50	20	11	104
	$\chi^2 = 11.736^*$ d.f. = 4 p .02–.01					

C. Anti-DNAse

Sex	Titer					Total
	<20	20–40	60–120	160–320	400–1280	
M	7	14	13	4	1	39
F	7	21	20	12	3	63
M & F	14	35	33	16	4	102
	$\chi^2 = 2.369$ d.f. = 4 p .70–.50					

than 160 units are comparable to those greater than 200 units of ASO.[21]

The anti-DNase B titer was measured by a modification[20] of the method of Wannamaker.[27] The distributions of these titers in post-streptococcal diseases and in some normal populations have been described.[22, 27] Titers of 320 units per ml of anti-DNase B are approximately comparable to 125 units of ASO and 80 units of anti-DPNase.

The distribution by sex of each of the three streptococcal antibody titers studied is shown in Table 7. Of the population studied 83% had ASO titers less than 200 units per ml; only one patient was observed to have a titer (625 units per ml) of the magnitude usually associated with recent infection. The anti-DNase B titers followed a pattern similar to that of ASO but with a somewhat wider distribution of their range. Of the anti-DNase B titers 90% were less than 640 units per ml (within the "normal" range for a U. S. population), the remainder being moderately increased (640 to 1280 units per ml). No titers exceeded 1,280 units per ml. The anti-DPNase titers were similar in distribution to those of anti-DNase B and antistreptolysin O. Eighty percent of anti-DPNase levels were less than 160 units per ml (within the U. S. "normal"); 16% were 160 to 320 units (moderately increased) and only 4% were at the level usually taken to indicate recent streptococcal infection. None exceeded 1,280 units per ml.

Because so few children were included in these samples, the data have not been analyzed for an age effect. However, a contrast by chi-square of the distribution of titers for males and females suggests that females were characterized by higher titers for antistreptolysin O ($\chi^2 = 9.277$, d. f. = 3, $.05 > p > .02$), but males, higher titers for anti-DNAse B ($\chi^2 = 11.736$, d. f. = 4, $.02 > p > .01$); there was no difference for anti-NADase. On the basis of a U. S. experience, the distribution of streptococcal-antibody titers in this population suggests sporadic exposure to mild streptococcal infection of some type.

ANTIBODIES TO THE POLIOMYELITIS VIRUS

The poliomyelitis virus may be taken as representative of a large group of enteric viral pathogens. Neutralizing-antibody titers against types I, II, and III of the virus were determined by the metabolic inhibition test in cultures of primary monkey-kidney cells.[28] The plasma specimens were first converted to serum as described earlier. Table 8 shows the frequency of positive tests to the three types of poliovirus by sex and village. The previously noted very high frequency of positive responses in the Xavante village near São Domingos[1] is confirmed, the percentage ranging from 71 to 95. Table 9 presents the results of a χ^2 analysis employing the arc-sine transformation of the data of Table 8 combined with the previously published data from a third Xavante village.[1] The analysis includes a partitioning into the three standard age groupings; 34.1% of the 314 specimens tested were from children less than 15 years of age. For all three virus types there is significant heterogeneity with respect to the cells of the age-sex-village breakdown. There

TABLE 8

*Frequency of occurrence of antibodies to poliovirus types I, II, and III in two Xavante villages**

Poliovirus type	Sex of those tested	Village					
		Simões Lopes			São Marcos		
		Total	Positive		Total	Positive	
			(*No.*)	(%)		(*No.*)	(%)
I	M	42	38	90.5	84	73	86.9
	F	31	29	93.5	97	74	76.3
	M & F	73	67	91.8	181	147	81.2
II	M	42	40	95.2	84	79	94.0
	F	31	29	93.5	97	91	93.8
	M & F	73	69	94.5	181	170	93.9
III	M	42	28	66.7	84	61	72.6
	F	31	27	87.1	97	67	69.1
	M & F	73	55	75.3	181	128	70.7

* The virus-neutralization technique was employed; only sera neutralizing at a dilution of 8 or more were scored as positive.

TABLE 9

Results of a χ^2 analysis, employing the arc-sine transformation, of percentage of positive reactions by age, sex, and village, to three strains of poliomyelitis virus

Source	Poliovirus I			Poliovirus II			Poliovirus III		
	χ^2	df	P	χ^2	df	P	χ^2	df	P
Village	8.022	2	.02–.01	2.102	2	.50–.30	13.818	2	<.001
Sex	7.287	1	.01–.001	0.120	1	.80–.70	0.129	1	.80–.70
Age	0.412	2	.90–.80	3.171	2	.30–.20	3.116	2	.30–.20
Interaction	20.806	12	.10–.05	23.427	12	.05–.02	83.839	12	<.001
Total	36.527	17	.01–.001	28.820	17	.05–.02	100.902	17	<.001

are village differences with respect to types I and III poliovirus; sex differences exist only for type I. The interaction term makes a significant contribution to the variability: the kinds of variation encountered are inconstant from village to village and age group to age group, especially as regards type III. This same finding was encountered, albeit to a lesser degree, with respect to the *Salmonella* antigens.

Comparison of the data of Table 8 with those previously published reveals a lower frequency of positive tests for all three virus types in São Domingos than in the other two villages. But since the tests that defined this difference were separated by an interval of 2 years, technical factors may be influencing the picture. From the epidemiologic standpoint, the important fact is not the difference but the remarkably high frequency of positive responses in all three villages. Crude though the age breakdown is, the absence of any suggestion of an age trend indicates early acquisition of the antibodies in question.

A separate χ^2 analysis, also employing the arc–sine transformation, of the data in Table 8 reveals that the observed lower frequency of positive responses to type III virus than to type I or II in the two villages is highly significant. This was also true in São Domingos.[1]

ANTIBODIES TO THE MEASLES VIRUS

Twenty-eight plasma specimens obtained in São Marcos were tested for the occurrence of

antibodies to measles virus by a modification of the fluorescent-antibody technique developed by Riggs and Brown[29, 30] and Brown *et al.*[31] for the detection of antibodies to the poliomyelitis virus. Twenty-five of these specimens gave 2, 3, or 4 plus reactions at a titer of 8 or greater, and are considered positive. A similar high frequency of positives was encountered in the Xavante village near São Domingos.[1] In an earlier paper[3] we discussed the possibility that in Simões Lopes infant mortality had increased recently, in part because of the introduction of measles. We must now recognize this possibility for São Marcos. To the extent that measles is a recent introduction, the argument[3] that before contacts with Western civilization mortality among infants and children in such groups was "intermediate" (as viewed on a world-wide scale) is strengthened.

ANTIBODIES TO THE ARBOVIRUSES

The presence of antibodies against various of the arboviruses was investigated with hemagglutination-inhibition tests, as described by Shope and Causey.[32] Twenty-three different virus antigens were employed as follows: *Group A*—1) Eastern equine encephalitis, 2) Aurá, 3) Mucambo, 4) Mayaro, 5) Una, and 6) Pixuna; *Group B*—7) yellow fever, 8) Ilhéus, 9) St. Louis, and 10) Bussuquara; *Group C*—11) Caraparú and 12) Itaquí; *Group Guamá*—13) Guamá, 14) Catú, and 15) Mojú; *Capim Complex*—16) Capim; *Phlebotomus-fever group*—17) Icoarací, 18) Bujarú, and 19) Itaporanga; *Turlock group*—20) Turlock; *Bunyamwera group*—21) Guaroa, and 22) Maguari; and 23) Taciuma, which is ungrouped. All of the viruses were isolated in Brazil. A response was recorded as positive if the specimen caused the inhibition of 8 units of antigen at a dilution of 1:20 or higher.

The principal results are shown in Table 10. Cross-reactions were observed within groups A, B, C, and Guamá. Among group A viruses the reactions were nearly all in highest titer to Mayaro. Plasma entered as positive under "Group B-Ilhéus" and "Group B-yellow fever" reacted primarily with the respective Group B component, whereas those entered under "Group B-Multiple" showed marked cross-reactions in Group B and probably indicate multiple Group B infections. The results on the two Bunyamwera group viruses are indicated separately, while the final entry is for the Group C and Guamá viruses. No plasma was positive to the Capim, Icoarací, Anhanga, Turlock, and Itaporanga viruses.

Table 11 presents an analysis of Table 10. Although not shown in Table 10, age was included as a parameter in the analysis (0–14, 15–30, and over 30 years); there is a significant age effect, the younger persons being less frequently positive. Further, as is apparent by inspection and corroborated by the high χ^2 value, there were great differences in the frequency of positive responses to various viruses and virus groups. Of the 412 specimens tested, 43% yielded no antibodies. Of those reacting with the Group A viruses, the titer was usually highest for Mayaro. Thirty-two specimens of serum were selected for neutralization tests on baby mice inoculated intraperitoneally with Mayaro virus. Sixteen of these had previously been positive to the Group A-Mayaro pool by HI testing; all but one of these gave a positive neutralization test (virus test dosage was 3.3 LD_{50}). Seven plasmas completely negative for antibody to Group A viruses by HI testing were also negative by neutralization test. Finally, of nine plasmas that inhibited Mayaro virus at the 1:10 but not the 1:20 dilution (and scored as negative), four neutralized 3.3 log LD_{50} of virus. This suggests not only that the principal agent eliciting the Group A antibodies is the Mayaro virus, but also that the criteria for a positive HI test have been conservative, and the true frequency of positives for Mayaro antibody is higher than indicated in the table. In addition, neutralization tests by intracerebral inoculation of baby mice demonstrated that several plasmas neutralized greater than 100 LD_{50} of the yellow-fever or Ilhéus viruses.

It is apparent that this population is extensively exposed to members of the arbovirus family. The presence of antibodies to the Mayaro virus in this group extends the known distribution to the southeast, while the occurrence of antibodies to the Ilhéus virus is characteristic of the Amazonas-Bahia Region and extends the distribution of this virus southward and westward. Since yellow-fever vaccination has not been carried out in the area, the presence of yellow-fever antibodies probably indicates natural yellow-fever infection in this population.

TABLE 10

Results of tests for antibodies to various types and groups of the arboviruses

Sex	Group A—Mayaro virus						Group B—Ilhéus					
	Simões Lopes			São Marcos			Simões Lopes			São Marcos		
	Total	Positive		Total	Positive		Total	Positive		Total	Positive	
		(No.)	(%)		(No.)	(%)		(No.)	(%)		(No.)	(%)
M	88	16	18.2	142	22	15.5	88	19	21.6	142	33	23.2
F	67	15	22.4	115	23	20.0	67	7	10.5	115	19	16.5
M & F	155	31	20.0	257	45	17.5	155	26	16.8	257	52	20.2
	Group B—yellow fever						Group B—multiple Group B					
	Simões Lopes			São Marcos			Simões Lopes			São Marcos		
	Total	Positive		Total	Positive		Total	Positive		Total	Positive	
		(No.)	(%)		(No.)	(%)		(No.)	(%)		(No.)	(%)
M	88	4	4.6	142	3	2.1	88	17	19.3	142	22	15.5
F	67	1	1.5	115	7	6.1	67	12	17.9	115	21	18.3
M & F	155	5	3.2	257	10	3.9	155	29	18.7	257	43	16.7
	Bunyamwera Group—Maguari						Bunyamwera Group—Guaroa					
	Simões Lopes			São Marcos			Simões Lopes			São Marcos		
	Total	Positive		Total	Positive		Total	Positive		Total	Positive	
		(No.)	(%)		(No.)	(%)		(No.)	(%)		(No.)	(%)
M	88	1	1.1	142	2	1.4	88	0	0	142	2	1.4
F	67	1	1.5	115	5	4.4	67	0	0	115	5	4.4
M & F	155	2	1.3	257	7	2.7	155	0	0	257	7	2.7
	Phlebotomus-fever Group—Bujarú						Group C and Guamá					
	Simões Lopes			São Marcos			Simões Lopes			São Marcos		
	Total	Positive		Total	Positive		Total	Positive		Total	Positive	
		(No.)	(%)		(No.)	(%)		(No.)	(%)		(No.)	(%)
M	88	0	0	142	2	1.4	88	1	1.1	140	4	2.9
F	67	2	3.0	115	3	2.6	67	1	1.5	114	3	2.6
M & F	155	2	1.3	257	5	1.9	155	2	1.3	254	7	2.8

DISCUSSION

Serologic epidemiology is still a rapidly evolving field—one must be extremely cautious in extrapolating from data such as these to agents and type of clinical disease. With all due reservations, then, the present data are compatible with this population's having experienced rather extensive contacts with at least nine categories of potentially disease-producing infectious agents: *Histoplasma*, *Toxoplasma*, *Plasmodia*, the *Salmonelleae*, *B. pertussis*, *Streptococci*, and certain types of virus: the enteric (poliomyelitis),

TABLE 11

Results of a χ^2 analysis, employing the arc-sine transformation, of percentage of positive reactions by age, sex, and village, for various groups of arboviruses

Source	χ^2	df	P
Village	0.649	1	.50–.30
Sex	0.257	1	.70–.50
Age	45.807	2	<.001
Pool type	266.760	7	<.001
Interaction	12.557	84	>.99
Total	326.030	95	<.001

respiratory (measles), and arbovirus groups. A previous paper has established that there has been extensive exposure to another category of potentially disease-producing agents, the intestinal parasites, ranging from *Entamoeba histolytica* to *Ascaris*.[33] There is no evidence for contact with tuberculosis or syphilis. The duration of the exposure to the pertussis, malaria, measles, and streptococcus organisms is most uncertain. Thus, while it seems clear that these latter organisms are often introduced to primitive groups by visitors from civilization, it is difficult, for example, to prove that the streptococcus did not accompany the Indian to the Americas, persisting ever since. However, it does seem clear that a high proportion in these two villages have had sufficient exposure to some of the diseases we associate with civilization so that infant and child mortality has probably increased in recent years—despite which relatively low values (by Tropical standards) were observed.[3] Thus, serologic epidemiology becomes an important adjunct to our efforts to understand the breeding structure of primitive man, which structure sets the boundaries within which natural selection has had to operate.[3, 34]

One of the prime challenges to population genetics is the explanation of the factors maintaining the many genetic polymorphisms present in all human populations thus far studied.[35] The conditions under which those polymorphisms became established are much more similar to those obtaining among the Xavante than among more civilized groups. In view of the interest in the possible relation between specific polymorphisms and specific disease, an attempt to determine the significant transmissible causes of morbidity and mortality at this cultural level has obvious genetic as well as epidemiologic interest. Although it may be possible, at this cultural level, to assemble sufficient numbers to establish that a particular genetic system is under selective pressure, it seems unlikely that it will be feasible to collect a sufficiently large series to test for relations between specific genetic systems and specific agents of disease.

SUMMARY

Varying numbers of Xavante Indians from two villages in the state of Mato Grosso in Brazil were tested for immunologic status with respect to various diseases and organisms. Among the findings of note were:

1. Positive skin tests to the histoplasmin antigen were encountered in 42.7% of those tested, but there were no certain positives for tuberculin or coccidioimycin.

2. All persons investigated gave positive tests for antibodies to *Toxoplasma*, usually in high titer.

3. Of those tested, 62% had antibodies to malaria antigens, usually in intermediate titers.

4. There was no serologic evidence (in a small series) for treponemal infections.

5. Between 30 and 80% of those tested, depending on the *Salmonella* subtype, had antibodies to *Salmonella* representative of groups A, B, C, and D.

6. Of those tested, 58% were found to have antibodies to *Bordetella pertussis*.

7. The distribution of titers to three streptococcal antigens suggests moderate contact with this pathogen.

8. Between 71 and 95% of subjects, depending on the specific type, had antibodies to poliomyelitis types I, II, and III.

9. Antibodies to measles were found in 89% of a small series.

10. The results of tests for antibodies against 23 arboviruses isolated in Brazil suggest extensive exposure to the Mayaro and Ilhéus viruses, and lesser exposure to a number of others, including yellow fever.

Although these findings constitute only a

beginning in defining the antibody profile among the Xavante, it is clear that this apparently healthy population has been exposed to a wide variety of what would ordinarily be termed pathogens.

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