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PRESUMPTIVE FALSE POSITIVE SEROLOGIC REACTIONS FOR SYPHILIS IN CENTRAL AMERICA

I. Incidence and Distribution

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THE PROBLEM of biologic false positive reactions in serologic tests for syphilis has assumed increasing importance during the last ten years due to: (1) growing recognition of the existence of these reactions, (2) more general use of blood tests for syphilis, (3) increased sensitivity and specificity of new and existing methods, and (4) a decrease in the incidence of syphilis in certain areas.

The biologic false positive potential becomes more important with the decrease of positive reactions due to syphilitic patients.[1] The status of biologic false positive reactions has been reviewed comprehensively by Beerman,[2] Davis,[3] Mohr, Moore, and Eagle,[4,5] Stokes,[6] Rein and Elsberg,[7] and others. During World War II, definite rules had to be formulated for the handling of personnel in the Armed Forces who gave doubtful or positive blood tests without clinical evidence of syphilis. It was noted in particular that military personnel returning from tropical areas were apt to have false positive reactions due to diseases such as malaria, filariasis, kala azar, etc. However, despite numerous investigations, there is still no definite knowledge as to the mechanism responsible for the production of biologic false positive reactions and with the possible exception of the Nelson treponemal immobilization test,[8] there is no absolute method for differentiating true and false positive reactions.

Biologic false positive reactions are, in general, divided into those associated with specific diseases other than syphilis, and those occurring in apparently normal individuals.[9] The first category does not usually present a serious diagnostic problem. The serologic results are associated with a definite clinical entity, in which a reversal to negativity occurs in a relatively short period of

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time. In the second group, the reactions fluctuate among positive, weak positive, and negative, and occur in apparently healthy individuals. Although certain persons seem more likely to develop false positive reactions than others, it is not known whether this is "due to (a) the presence of antibody-like substances similar to antibodies produced in syphilitic diseases; (b) an increase or alteration of the sero-globulin fraction or; (c) an increase or alterations of some chemical substance or substances in the blood."[10]

In 1946, a Venereal Disease Research Laboratory was established in Guatemala City, Guatemala, in cooperation with the Ministry of Health of Guatemala, by the Pan American Sanitary Bureau, with the assistance of the United States Public Health Service. Preliminary surveys[11] demonstrated that a high incidence of positive and doubtful reactions were obtained when tests using lipoidal antigens (Kahn, Eagle, Mazzini, and Hinton) were used, while the tests employing cardiolipin antigens (VDRL, Kline, Rein-Bossak, and Hinton cardiolipin) gave a much lower reactivity and were more in agreement with the clinical findings. This was also true of the Kolmer complement fixation tests with either the lipoidal or cardiolipin antigen.

In order to investigate the relation between the clinical and serologic findings, a study was conducted among a group of 515 children in an orphanage in Guatemala City.[12] Positive and doubtful reactions with the Kahn and Mazzini tests were 26.4 per cent and 34.4 per cent respectively, while the VDRL test showed 2.6 per cent reactions, and the Kolmer complement fixation test 3 per cent. Only three children in this group were diagnosed as having congenital syphilis. It was concluded[12] that the serologic reactions with the tests employing lipoidal antigens did not necessarily represent syphilis. These false positive findings could not be accounted for by the results of clinical examination.

A similar study[13] was conducted among the school children of San José, Guatemala, where malaria is endemic. Although malaria is a well known cause of false positive reactions, the authors concluded that this would not account for the high percentage of positive and doubtful reactions which occurred in this group with the Kahn and Mazzini tests.

Surveys made in Guatemala in 1948[14] on groups of Indian and Ladino* children showed similar patterns of reactivity, with 10 to 23 per cent doubtful and positive reactions with the Kahn and Mazzini tests, and only 1 to 2 per cent reactions with the VDRL and Kolmer tests. In random adult groups of both Indians and Ladinos, approximately twice as many reactions were obtained with the Kahn and Mazzini tests using lipoidal antigens as with the Kolmer and VDRL tests. In El Salvador and Costa Rica, as well as Guatemala, a significant difference in reactivity was shown to occur between the lipoidal tests (Kahn, Eagle) and the VDRL test.

The World Health Organization Expert Committee on Venereal Infections,[11] in the report of its Subcommittee on Serology and Laboratory Aspects, stated "there is some evidence to support the belief that the prevalence of the disease (syphilis) in some world areas has been greatly exaggerated, being based on the high proportion of positive serologic findings. It is very possible that this pic-

*Mixed European-Indian descent.

ture is the result of an environmental condition quite distinct from syphilis." Mahoney and Zwally[16] also mention the extraordinarily high incidence of positive findings which have been observed in some tropical areas and draw the inference that "unrecognized environmental factors may be responsible for a serologic picture entirely at variance with the frequency with which syphilis is encountered."

Parasitic infestation, racial differences, dietary factors, and many other possibilities have been suggested to explain the relatively high incidence of presumptive false positive reactors in the Central American area. However, no systematic study has been reported of any of these factors in relation to this problem. In a series of four papers, we report investigation of certain specific factors in relation to biologic false positive reactions.

In the present paper, the distribution of serologic reactions in tests for syphilis among representative groups of school children in Costa Rica, El Salvador, Guatemala, and Honduras is reported. The effect of school feeding combined with parasite treatment in Guatemala is considered. The second paper in the series discusses the relation of these reactions to certain serum vitamin levels; the third, the correlation with serum protein, albumin, and globulin. The fourth in this series reports their relation to the results of the cephalin cholesterol (Hanger) flocculation test in these groups.

MATERIAL AND METHODS

School children, ages 7 to 12 inclusive, from four Central American countries were studied. In Guatemala, five rural schools were represented; in El Salvador, three urban and three rural schools; and in Honduras, four urban schools. The Costa Rican children included in the survey attended school in the capital city of San José. All were given careful physical examinations and appeared free of signs of congenital syphilis. Only one of the schools—a rural school in El Salvador—was in a malarious area.

These children were in general markedly underdeveloped by European standards. Although the retardation is believed due at least in part to poor nutrition, this cannot be stated with certainty in the absence of adequate growth studies for well nourished children of these racial groups. The only clinical finding suggestive of vitamin deficiency was the frequent occurrence of follicular hyperkeratosis. This finding is frequently, but not always, related to vitamin A deficiency.[17]

For the supplementary feeding program in Guatemala, the children received a midmorning "snack" which included a glass of milk* in one school, and its equivalent in a vegetable protein drink (usually made with soy bean powder) in another. Additional seasonal vegetables, fruits, and tortillas (flat cakes made from ground corn previously soaked in lime water) were provided. Approximately 90 per cent of the children were initially infected with *Ascaris lumbricoides*; and many also carried *Trichiuris trichiura* and various other parasites. All received the standard 1 per cent chenopodium preparation provided by the

*The milk was furnished without charge by the United Nations International Children's Endowment Fund and the soy bean preparation by the Soya Food Research Council of the United States.

Guatemalan Health Department for the treatment of intestinal parasites, and the degree of infestation as disclosed by stool examinations was temporarily reduced.

The serologic testing was done in a modern, well equipped laboratory. All testing procedures were performed as described in the 1949 Manual of Serologic Tests for Syphilis.[18]

A battery of five tests was used. The Kahn and Mazzini tests were used as representative of the older tests using lipoidal antigens; the VDRL and Kline tests represented slide tests using antigens composed of cardiolipin, lecithin, and cholesterol. The Kolmer test represents the complement fixation reactions, using a lipoidal antigen.

All the antigens used in this study were prepared and furnished by courtesy of the Venereal Disease Research Laboratory, USPHS, with the exception of the Kline cardiolipin antigen which was purchased from the La Motte Chemical Company, Baltimore, Maryland. The statistical tests were carried out by standard methods.[19,20]

CLASSIFICATION OF SEROLOGIC REACTIONS

Group I. Doubtful Reactors.—These include reactions doubtful in Kahn and/or Mazzini or positive in one but not in both of these tests. All in this group were negative with the Kolmer, VDRL, and Kline tests. When this classification was established, it was not known whether this group constituted biologic false positive reactions or a doubtful group of no special significance.

Group II. Presumptive False Positive Reactors.—This group includes specimens which react positively in both the Kahn and Mazzini tests, and those which gave positive reactions with one test and doubtful with the other. All the specimens were negative with the Kolmer, Kline, and VDRL tests. These reactions are considered presumptive biologic false positive reactions.

Group III. Presumptive Syphilitic Reactors.—On the basis of positive and doubtful reactions with all the testing procedures, Kahn, Mazzini, VDRL, Kline, and Kolmer, this group is considered syphilitic. Nevertheless, this category may also contain some biologic false positive reactors, since it is well known that nonspecific reactions do occur in the cardiolipin[21,22] and Kolmer[23] tests. This group is not important numerically and is omitted from the statistical comparisons.

Group IV. Negative Reactors.—Negative reactions were obtained in all five tests for syphilis.

RESULTS

The incidence of positive, doubtful and negative reactions as determined by the Kahn, Mazzini, Kolmer, VDRL, and Kline tests in four Central American countries is shown in Table I. It will be noted that both the Kahn and Mazzini tests give a high percentage of positive and doubtful reactions. On the other

hand the Kolmer, VDRL, and Kline tests show a lower percentage of reactions more compatible with the expected incidence of congenital syphilis in this group.

TABLE I. COMPARISON OF THE REACTIVITY OF SEROLOGIC TESTS FOR SYPHILIS IN CENTRAL AMERICA

	KAHN		MAZZINI		KOLMER		VDRL		KLINE	
	NUMBER OBSERVED	PER CENT	NUMBER OBSERVED	PER CENT	NUMBER OBSERVED	PER CENT	NUMBER OBSERVED	PER CENT	NUMBER OBSERVED	PER CENT
Positive	156	9.1	272	14.0	37	2.4	34	1.7	32	1.7
Doubtful	141	8.3	372	19.2	7	0.4	17	0.9	27	1.4
Negative	1411	82.6	1297	66.8	1507	97.2	1916	97.4	1855	96.9
Total	1708	100.0	1941	100.0	1551	100.0	1967	100.0	1914	100.0

Table II shows a comparison of the distribution of serologic reactions in Costa Rica, Guatemala, El Salvador, and Honduras in accordance with the classification described. No significant difference is noted among the countries.

TABLE II. DISTRIBUTION OF SEROLOGIC REACTION TYPES IN CENTRAL AMERICA

	COSTA RICA		EL SALVADOR		GUATEMALA		HONDURAS		TOTAL	
	NUMBER OBSERVED	PER CENT	NUMBER OBSERVED	PER CENT	NUMBER OBSERVED	PER CENT	NUMBER OBSERVED	PER CENT	NUMBER OBSERVED	PER CENT
Group I	67	23	16	27	24	24	17	28	124	24
Group II	11	4	3	5	9	9	8	13	31	6
Group III	11	4	11	19	4	4	1	2	27	5
Group IV	200	69	29	49	64	63	35	57	328	65
Total	289	100.0	59	100.0	101	100.0	61	100.0	510	100.0

A comparison was also made of serologic reactivity in groups of children before and after the combined supplementary feeding and parasite treatment program, as shown in Table III. No essential change was noted.

TABLE III. THE EFFECT OF FEEDING AND INTESTINAL PARASITE TREATMENT ON THE DISTRIBUTION OF REACTOR GROUPS IN GUATEMALA

	INITIAL SURVEY		RESURVEY IN 1 YEAR		
	NUMBER OBSERVED	PER CENT	NUMBER OBSERVED	PER CENT	X^2
Group I	15	23.8	12	27.3	.219
Group II	9	14.3	7	15.9	.020
Group IV	39	61.9	25	56.8	.186
Total	63	100	44	100	.485*

*For the "Chi square" value cited and two degrees of freedom, the probability is .80

DISCUSSION

Results similar to those of the orphanage[11,12] were found in this study. The material presented indicates that there is a wide variation between the reactivity of the tests for syphilis, which use lipoidal antigens (Kahn and Mazzini), and the tests which use cardiolipin antigens (VDRL and Kline). The latter tests are in fairly close agreement with the complement fixation tests (Kolmer). There is no clinical evidence which would indicate that a high incidence of congenital or latent acquired syphilis is present among these groups of school children. The presumptive evidence is in favor of this reactivity representing biologic false positive reactions of unknown etiology. This was recognized by Mahoney,[23] who says, "A very general cause other than syphilis, for the high prevalence of positive findings appears to be operative in the American Indian, the Negro race, the laboring class in Mexico, and the Indian in Guatemala."

It is apparent from the data presented that the school feeding (combined with parasite treatment) did not significantly influence the reactivity. This is in accord with the results of Breazeale, referred to by McCammon and associates,[24] who was unable to relate nutritional habits to unsatisfactory results in flocculation tests for syphilis in groups of Pima, Papago, Navaho, and Hopi Indians in the southwest. On the other hand, the report of Barnes and his co-workers[25] that biologic false positive serologic reactions tend to disappear with a change to a low protein diet is difficult to interpret in the light of the present findings. The people in the rural areas, especially in Guatemala, are living on a diet exceedingly low in protein of good quality. Thus they should have a low incidence of biologic false positive reactions, according to Barnes' findings, and not a high one.

The present report does not constitute proof that the serologic reactions observed may not still be associated with heavy infestation of intestinal parasites. In the cases presented, prompt reinfection, as well as incomplete cures, prevented satisfactory elimination of infestation. Moreover, two variables were introduced simultaneously, parasite treatment and supplementary feeding. Serologic changes resulting from the presence of parasites might be also expected

to persist long after the infestation had been eliminated. It would seem that the relation to parasitic disease should still be explored although the failure to observe any change in this series does not encourage the hypothesis that nutritional or parasitic factors are related to the biologic false positive phenomenon.

SUMMARY

Over 1500 school children in Costa Rica, El Salvador, Guatemala, and Honduras were examined for serologic reactivity with the Kahn, Mazzini, Kolmer, VDRL, and Kline tests for syphilis. Twenty-four per cent were classified in a doubtful reactor group; 6 per cent as presumptive false positive and 5 per cent as possible syphilis. The percentages of doubtful reactors varied from 23 per cent to 28 per cent and presumptive false positives from 4 per cent to 13 per cent among the four countries.

The percentage of presumptive false positive reactors was not changed as a result of combined supplementary school feeding and treatment for intestinal parasites, under conditions of prompt reinfestation.

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