

## A Study of Enteric Fever Agglutinins in Ceylon\*

by

T. VELAUDAPILLAI AND L. A. ALPIN SINGHO

*Medical Research Institute, Colombo*

Clinicians and those associated with them are familiar with the term "Standard Agglutination Test" hereafter referred to as S.A.T. in this paper. The presence of antibodies due to *Salmonella typhi* and other allied organisms in inoculated and uninoculated persons and in enteric fever cases has been studied by many workers from 1930-1945. For example, studies have been reported from the United Kingdom (Smith, 1932), Canada (Wyllie, 1932), Sudan (Horgan, 1932), West Indies (Giglioli, 1933) and India (Wilson, 1945). Although typhoid fever is prevalent throughout Ceylon, similar reports have not been published in this country. Results of such studies will be useful to clinicians in interpreting S.A.T. results in patients specially in those who may have received T.A.B. inoculations sometime earlier.

This paper gives the results of studies of S.A.T. done on sera of inoculated and uninoculated persons and a few cases of continued fever.

### MATERIALS AND METHODS

Samples of bloods from donors at the Blood Bank, General Hospital, Colombo (G.H.C.), and from patients attending the Venereal Diseases Clinic (V.D.C.), Colombo were examined. The specimens were collected at random and the survey was carried out from 27th April, 1964 to 13th January, 1965. Particulars such as age, residence and occupation of the persons, whether they had typhoid fever or continued fever of at least ten days' duration within the last three years and details of T. A. B. inoculations if any were collected at the time the blood was drawn. Standard methods (Report, 1961) were used for the agglutination tests which were done up to a maximum titre of 1:200 with *Salm. typhi* H and O and *Salm. paratyphi A* H antigens. *Salm. typhi* Vi agglutination tests were done in dilutions of 1:10. The blood clots were cultured in ox bile. During the final stages of the work, whenever agglutinations were positive, titrations were done up to the end points (fading titres).

### RESULTS AND DISCUSSIONS

Tables I and II show the incidence of agglutinins among non-inoculated and inoculated persons who gave no history of continued fever of at least ten days' duration within the last three years.

\* Paper read at the 21st Annual Session of the Ceylon Association for the Advancement of Science.

TABLE I  
*Agglutinins in Non-inoculated People*

Antigen	Number of sera agglutinating in dilutions of					Total positives
	Negative	1:50	1:100	1:200	1:400	
<i>Salm. typhi</i> O	990	11 (1.1)	7 (0.7)	—	—	18 (1.8)
<i>Salm. typhi</i> H	901	52 (5.2)	33 (3.3)	22 (2.2)	—	107 (10.6)
<i>Salm. typhi</i> Vi	1006	—	—	—	—	2 (0.2)
<i>Salm. paratyphi</i> A H	984	10 (1.0)	9 (0.9)	4 (0.4)	1 (0.1)	24 (2.4)

Percentages are given in brackets.

A total of 1008 samples of blood from uninoculated persons were examined. Of these 18 (1.8%) samples had *Salm. typhi* O agglutination titres of 1:50 and 1:100. The percentage of positives for *Salm. typhi* H and *Salm. paratyphi* A H were 10.6 and 2.4 respectively.

TABLE II  
*Agglutinins in Inoculated People*

Antigen	Number of sera agglutinating in dilutions of					Total positives
	Negative	1:50	1:100	1:200	1:400	
<i>Salm. typhi</i> O	74	7 (8.3)	2 (2.4)	1 (1.2)	—	10 (11.9)
<i>Salm. typhi</i> H	50	5 (6.0)	10 (11.9)	19 (22.6)	—	34 (40.5)
<i>Salm. typhi</i> Vi	83	—	—	—	—	1 (1.2)
<i>Salm. paratyphi</i> A H	63	7 (8.3)	7 (8.3)	4 (4.8)	3 (3.6)	21 (25.0)

Percentages are given in brackets.

Agglutination tests were done with 84 sera of inoculated persons. The percentages of sera giving positive reactions were considerably higher than those of the uninoculated group. About one third of the samples were positive for both *Salm. typhi* H and *Salm. paratyphi* A H among those who received T.A.B. inoculations, whereas similar positive results for the non-inoculated persons were to the extent of 12%. A few samples from

both groups were positive reactions with *Salm.typhi* O and H antigens and some with *Salm.typhi* O and H and *Salm. paratyphi A* H antigens. Some of these results will be shown later in Table VII. As a certain proportion of sera from uninoculated people gave positive reactions, inquiries were made a second time to find out whether they were previously inoculated or had typhoid fever or had continued fever of at least ten days' duration before or after drawing the blood. The replies confirmed the history already given at the times the bloods were collected.

Table III shows the prevalence of antibodies in the sera of venereal diseases patients and blood donors.

TABLE III  
*Agglutinins in Sera of Persons According to the Institution Attended*

Institutions	No. T.A.B.			T.A.B.			Old typhoid cases			Total		
	+ & %	0	Total	+ & %	0	Total	+ & %	0	Total	+ & %	0	Total
V.D.C., Colombo	30 (14.0)	186	216	9 (45.0)	11	20	2 (50.0)	2	4	41 (17.1)	199	240
Blood Bank, G.H.C.	98 (12.4)	694	792	28 (43.8)	36	64	2 (50.0)	2	4	128 (14.9)	732	860
Total	128 (12.7)	880	1008	37 (44.0)	47	84	4 (50.0)	4	8	169 (15.4)	931	1100

A serum reacting with one or more antigens was counted as one serological case.  
Percentages are given in brackets.

A total of the 240 samples of blood from venereal diseases patients and 860 from blood donors were examined. This table and the subsequent table divide the cases into three categories (1) those who had received no T.A.B. inoculations (2) those who had received the inoculations and (3) those who had typhoid fever previously.

Although most of the cases from the Venereal Diseases Clinic suffered from some type of venereal infection, the percentage of serologically positive cases among uninoculated venereal disease patients (14.0%) was not significantly different to uninoculated blood donors (12.4%). Even among the inoculated group there was no difference in the reactivity between the two classes of people. It appears that venereal diseases do not tend to increase the positive rates through anamnestic reaction. Hereafter, the tables will not draw any distinction between venereal diseases patients and blood donors.

Table IV shows the distribution of agglutinins according to age groups.

TABLE IV  
*Antibodies in Sera According to Age*

Age Group	No. T.A.B.			T.A.B.			Old typhoid cases			Total		
	+ & %	0	Total	+ & %	0	Total	+ & %	0	Total	+ & %	0	Total
11 — 19	3 (4.7)	61	64	1 (20.0)	4	5	—	—	—	4 (5.8)	65	69
20 — 29	47 (9.3)	458	505	18 (46.2)	21	39	1 (25.0)	2	3	66 (12.1)	481	547
30 — 39	61 (17.0)	298	359	14 (43.8)	18	32	3 (60.0)	2	5	78 (19.7)	318	396
40 — 49	17 (22.4)	59	76	4 (50.0)	4	8	—	—	—	21 (25.0)	65	84
50 — 59	—	2	2	—	—	—	—	—	—	—	2	2
60 — 69	—	2	2	—	—	—	—	—	—	—	2	2
Total	128 (12.7)	880	1008	37 (44.0)	47	84	4 (50.0)	4	8	169 (15.4)	931	1100

Percentages are given in brackets.

About half the number of cases were in the age group 20-29 and about 85% between the ages 20-39. With the exception of six female donors from the Blood Bank, all persons investigated were males. As the age increased, the percentage of people having antibodies also increased. Among the uninoculated, it was 4.7% for the 11-19 age group, and reached a maximum of 22.4% for the 40-49 age group. For the inoculated group, it began at 20.0% for 11-19 age group and ended up at 50.0% for the 40-49 age group. The number of cases studied for ages above 50 was too small and no comments are being made. It is not possible to give a satisfactory explanation for the high positive rate among the older uninoculated people. It is probable that some might have had subclinical infection or received T.A.B. inoculations.

In Table V, the agglutination results are analysed according to the provincial residence of the persons.

TABLE V

*Classification of Antibodies in People According to Their Provinces.*

Provinces (towns are given in brackets)	No. T.A.B.			T.A.B.			Old typhoid cases			Total						
	+	& %	0	Total	+	& %	0	Total	+	& %	0	Total				
Western (25)	112	(12.6)	769	881	32	(42.2)	42	74	4	(66.7)	2	6	148	(15.4)	813	961
Southern (12)	8	(16.0)	42	50	—	—	4	4	—	—	2	2	8	(14.3)	48	56
Sabaragamuwa (4)	2	(7.4)	25	27	2	—	—	2	—	—	—	—	4	(13.8)	25	29
Central (6)	4	(16.7)	20	24	—	—	1	1	—	—	—	—	4	(16.0)	21	25
North Western (4)	—	—	13	13	—	—	—	—	—	—	—	—	—	—	13	13
Northern (2)	1	(16.7)	5	6	1	—	—	1	—	—	—	—	2	(28.6)	5	7
North Central (4)	1	(25.0)	3	4	2	—	—	2	—	—	—	—	3	(50.0)	3	6
Uva (one)	—	—	3	3	—	—	—	—	—	—	—	—	—	—	3	3
Total (58)	128	(12.7)	880	1008	37	(44.0)	47	84	4	(50.0)	4	8	169	(15.4)	931	1100

Blood samples from persons residing in 58 villages or towns were examined but nearly all were resident at the Western Province. A reasonable number of blood samples from people in the Western, Southern, Sabaragamuwa and Uva Provinces were examined. The overall positive rates for those provinces were nearly the same.

Table VI shows the presence of antibodies in inoculated persons according to the period after T.A.B. inoculation.

When blood samples were tested within one year of inoculation, the positive rate was 75%; when tested between one and two years, it was over 60%. The rate remained high for the next two years. For those tested after 4 years and before 10 years, the positive rate was the same, but was lower than in the previous years. As the time after inoculation increased, the agglutinin titre decreased. Therefore, any information about when the patient was inoculated would be helpful in assessing the results.

TABLE VI  
Antibodies in Sera According to the Period after Inoculation

Period after Inoculation	Positive & %	Negative	Total
Less than one year	6 (75.0)	2	8
Over one year & less than 2 years	8 (61.5)	5	13
Over 2 years & less than 3 years	9 (47.4)	10	19
Over 3 years & less than 4 years	5 (45.5)	6	11
Over 4 years & less than 5 years	2 (28.6)	5	7
Over 5 years & less than 6 years	2 (25.0)	6	8
Over 6 years & less than 7 years	2 (33.3)	4	6
Over 7 years & less than 10 years	3 (25.0)	8	11
Over 10 years	—	1	1
Total	37 (44.0)	47	84

A few serological results with fairly high titres in inoculated and uninoculated persons without fever are scheduled in Table VII.

TABLE VII  
High Titre Agglutinins in Sera of Uninoculated and Inoculated Persons

Titres	No. with no T.A.B.	No. and period after inoculation
<i>S. typhi</i> O 1:100	5	1 (2 years)
<i>S. typhi</i> H 1:100; O 1:50	2	—
<i>S. typhi</i> H 1:100; O 1:100	2	—
<i>S. typhi</i> H 1:200; O 1:50	1	—
<i>S. typhi</i> H 1:200; O 1:100	—	1 (2 years)
<i>S. typhi</i> H 1:100; O 1:50; <i>S. paratyphi A</i> H 1:50	1	—
<i>S. typhi</i> H 1:100; O 1:50; <i>S. paratyphi A</i> H 1:100	—	1 (1 year)
<i>S. typhi</i> H 1:200; O 1:50; <i>S. paratyphi A</i> H 1:50	—	1 (1 year)
<i>S. typhi</i> H 1:200; O 1:50; <i>S. paratyphi A</i> H 1:50	—	1 (1½ years)
<i>S. typhi</i> H 1:200; O 1:100; <i>S. paratyphi A</i> H 1:400	—	1 (2 years)
<i>S. typhi</i> H 1:200; O 1:200; <i>S. paratyphi A</i> H 1:200	—	1 (2 months)
<i>S. typhi</i> H 1:200; O 1:50; <i>S. paratyphi A</i> H 1:400	—	1 (4 months)
<i>S. typhi</i> H 1:200; O 1:50; Vi; <i>S. paratyphi A</i> H 1:400	—	1 (1 year)
<i>S. typhi</i> H 1:1,600; <i>S. paratyphi A</i> H 1:200	—	1 (7 months)
<i>S. typhi</i> H 1:50; O 1:100; <i>S. paratyphi A</i> H 1:100	—	1 (1½ years)
<i>S. typhi</i> H 1:1,600; O 1:200; <i>S. paratyphi A</i> H 1:400	—	1 (1 month)

All the samples of blood except the last two were from the Blood Bank and Venereal Diseases Clinic, Colombo. The last two were from a junior Government Servant and a Naval Officer, neither of whom had fever at the time the blood was drawn. If these samples were collected when they had fever, the clinicians would have been misled by these results because the titres were high. However, the residual antibodies are not always high, and a repeat test will be useful in ascertaining the changing titres, provided the patients were not inoculated recently. To determine these values, the end point agglutinations will have to be done whenever agglutinations are positive. This procedure is now being followed as a routine at the Medical Research Institute, Colombo.

In Table VIII are presented the results of some of the clinically positive typhoid cases.

TABLE VIII  
*Serological Results in Some Known Typhoid Cases*

Case	Date	Serological results
M.F.	27.4.65	<i>S. typhi</i> O 1:400
	3.5.65	<i>S. typhi</i> O 1:200
V	19.5.65	<i>S. typhi</i> H 1:1,600; O 1:100
	24.5.65	<i>S. typhi</i> H 1:1,600; O 1:100
	9.6.65	<i>S. typhi</i> H 1:400; O 1:100
M.M.	10.6.65	<i>S. typhi</i> H 1:50; O 1:400
	16.6.65	<i>S. typhi</i> H 1:50; O 1:400
	18.6.65	<i>S. typhi</i> H 1:50; O 1:800
A.	26.2.65	<i>S. typhi</i> O 1:50; <i>S. typhi</i> from clot
	3.3.65	<i>S. typhi</i> O 1:200; H 1:400
G.	17.5.65	<i>S. typhi</i> H 1:100
	20.5.65	<i>S. typhi</i> H 1:400; <i>S. typhi</i> from clot
	24.5.65	<i>S. typhi</i> H 1:400; O 1:400
	29.5.65	<i>S. typhi</i> H 1:800; O 1:400; Vi-+
J.	9.7.65	<i>S. typhi</i> O 1:100; <i>S. typhi</i> from clot
	19.7.65	<i>S. typhi</i> O 1:100; H 1:200

It is well known that a serological test must be repeated when the first result does not give sufficient information. But under the old procedure, the tests would have been done to a maximum titre of 1:200 and in the first three cases, the laboratory results would then be the same. Clinicians would not have got extra assistance from the agglutination results even though the titres had really changed. However, if the end point agglutinations were done and the titres were high, the diagnosis would have been clear even with one test; a changing titre would also indicate infection. Therefore, it is essential for the laboratories doing S.A.T. to report the end points whenever the agglutinations are positive. At the Medical Research Institute, in some cases, titres as high as 1:25,600 were obtained for *Salm.typhi* H and 1:6,400 for *Salm.paratyphi* A H. *Salm.typhi* O titres were not high and did not exceed 1:1,600.

Some of the false positive results in fever cases are listed in Table IX.

TABLE IX  
False Positive Results

Case	Date	Serological results—Date of inoculation and Clinical Diagnosis
S.M.B.S.	29.4.65	<i>S. typhi</i> H 1:100; O 1:200; <i>S. paratyphi</i> A H 1:100
	3.5.65	<i>S. typhi</i> H 1:200; O 1:100; <i>S. paratyphi</i> A H 1:100 Inoculated on 16.2.65. Influenza.
H.A.N.	19.5.65	<i>S. typhi</i> H 1:3,200; O 1:100; <i>S. paratyphi</i> A H 1:1,600
	21.5.65	<i>S. typhi</i> H 1:1,600; O 1:100; <i>S. paratyphi</i> A H 1:3,200 Inoculated on 19.5.65. Pneumonia
D.D.J.	10.6.65	<i>S. typhi</i> H 1:800; O 1:400; <i>S. paratyphi</i> A H 1:800 Inoculated on 10.5.65. Influenza
K.	19.7.65	<i>S. typhi</i> H 1:200
	26.7.65	<i>S. typhi</i> H 1:800 Inoculated on 6.7.65. Filariasis.
R.M.	13.7.65	<i>S. typhi</i> O 1:100
	19.7.65	<i>S. typhi</i> O 1:100 Not inoculated. Non specific Entero-colitis.
S.	27.3.65	<i>S. typhi</i> O 1:100
	1.4.65	<i>S. typhi</i> O 1:100
	5.4.65	<i>S. typhi</i> O 1:100 Not inoculated. Infective Hepatitis.

Recent T.A.B. inoculations produced very high agglutination titres both for H and O. On repetition of the tests, the titres varied as in enteric infection. In these cases, S.A.T. will be of no value and the clinicians will have to depend on blood culture. At the M.R.I., clot culture is being done on all samples sent for S.A.T. In several cases where serological results were of doubtful value, *Salm.typhi* was isolated from the clots. It is suggested that all laboratories performing S.A.T., do clot culture also. With antibiotic treatment, typhoid patients stay for a short time in the hospitals, so a repeat S.A.T. will not always be possible. Another factor to be borne in mind is the issue of over 200,000 doses of T.A.B. vaccine per year. This means about a million people will be inoculated once in five years. Therefore it is likely that more and more false positives will be reported in future.

From one sample of blood agglutinating with *Salm.typhi* O antigen at a titre of 1:100, *Salm.enteritidis* was isolated. This is due to the fact that both these organisms have the same O antigen. On another occasion, *Salm.stanley* was isolated from a blood agglutinating with *Salm.typhi* H antigen in a dilution of 1:100. Flagellar antigen 'd' is possessed by those two organisms, so great care should be exercised in interpreting S.A.T. results in children as *Salm.stanley* is very frequently isolated from the faeces of children all over the island.

It is a well known fact that persons who have suffered from typhoid fever have antibodies in the blood for a long time. Unfortunately, in the present study, only eight cases were investigated. Of these, four had antibodies in their blood.

### SUMMARY

- (1) A random survey among blood donors at the General Hospital and male patients of the Venereal Diseases Clinic, Colombo, revealed that 15.4% of them had enteric fever antibodies. The rate was 12.7% among the uninoculated, and 44.0% among the inoculated.
- (2) In interpreting S.A.T. results, the clinicians should take into consideration the age of the patients, whether they had received T.A.B. inoculations and whether they had typhoid fever previously.
- (3) Laboratories performing S.A.T. should report end point agglutinations in positive cases.
- (4) These laboratories, in addition to S.A.T., should do clot culture.

### ACKNOWLEDGEMENTS

The Superintendents Anti Venereal Diseases Campaign and Blood Bank, General Hospital, Colombo are thanked for supplying samples of blood. The clinicians are also thanked for providing the clinical diagnosis of the patients. The help rendered by the Medical Statistician of the Health Department is gratefully acknowledged.

### REFERENCES

- GIGLIOLI, G. (1933). Agglutinins for typhoid-paratyphoid group in a random sample of the population of British Guiana. *J. Hyg.* 33, 379-386.
- HORGAN, E. S. (1932). Qualitative or quantitative methods in the serological diagnosis of enteric infections. *Ibid.* 32, 523-528.
- REPORT (1961). Serological reagents for the bacteriological diagnosis. *Mon. Bull. Minist. Hlth. Lab. Serv.* 20, 134-149.
- SMITH, J. (1932). The serological diagnosis of typhoid and paratyphoid fevers. *J. Hyg.* 32, 143-155.
- WILSON, J. F. (1945). Agglutination tests in the diagnosis of enteric fever in the inoculated. *Ibid.*, 44, 129-134.
- WYLLIE, J. (1932). Experiences with small flaking or granular agglutination in normal, inoculated and enteric fever cases. *Ibid.*, 32, 375-384.