

# Observations on the Diagnosis of Typhoid Fever in an Endemic Area

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## SUMMARY

The problem of providing laboratory confirmation of the diagnosis of typhoid in an endemic area was considered. A consecutive series of pyrexial patients was studied clinically, by blood and stool culture, the Widal reaction, the Sclavo rapid slide test and the diazo reaction. High Widal titres at even very early stages of typhoid suggested an anamnestic response. However, few non-typhoid cases had detectable Widal titres, and it seems, therefore, that though post-typhoid levels of antibody may be very low or even undetectable, further contact gives rise to a secondary response. Diagnostic criteria based upon a single Widal reading should be established for endemic areas, since rising titres may not be demonstrable. Though the rapid slide tests and the diazo reaction may be of some use in screening procedures in non-endemic areas, the results in endemic areas are likely to be confusing.

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The diagnosis of typhoid fever is likely to be more difficult in countries where the disease is endemic than in those in which cases occur either sporadically or in small outbreaks. In certain areas in Rhodesia enteric fever is endemic,<sup>1</sup> and under such circumstances the clinical presentations may differ quite markedly from the classical. Therefore considerable reliance must be put on the laboratory to confirm a clinical suspicion accurately and as early as possible. The available laboratory tests are isolation of the organism, the Widal test, rapid slide tests, and the more recently advocated diazo reaction on the urine. Isolation of the organism is obviously the most important diagnostic test, but isolation may be difficult. The results of the Widal tests are open to a wide variety of interpretations, which depend upon prevailing local conditions, such as the degree of endemicity, the extent of the carrier state, attack rate, and immunisation programmes. The diazo reaction has not been critically assessed as yet, but it seems to be on a rather non-specific basis.

The purpose of this article is to present certain laboratory data in respect of a consecutive series of pyrexial

patients who were prospectively reviewed after admission to hospital. Of these patients 20 were finally diagnosed as having typhoid fever.

## PATIENTS AND METHODS

During an 8-week period, 100 pyrexial Black patients (aged 7-60 years, 69 males) were admitted to the wards of Harare Hospital, Salisbury. Most came from Mashonaland, particularly from semirural and rural areas. No typhoid epidemics occurred during this period of study.

Of the 20 typhoid patients, the sex distribution was equal, and the majority of patients were under the age of 30 years.

**Isolations:** Standard techniques were employed for specimens from blood, faeces and urine. The media used were bile broth, McConkey's agar, DCA medium and selenite broth enrichment. Definitive identification was by serological methods.

**Widal tests:** Sera were screened initially against both H and O antigens at a dilution of 1 in 40. Only those positive at 1 in 40 were subsequently titrated to an end point. Thus titres of less than 1 in 40 are scored as negative. Sera from patients in the control group were examined only once, but those in whom the diagnosis of typhoid was pursued were, with 4 exceptions, examined on at least two occasions. The sera were all retested at a later date, at the same time and with the same batch of antigen.

**Diazo test:** The test was performed by placing approximately 5 ml of freshly voided urine into a test-tube and adding an equal volume of diazo solution. After mixing, 3 to 4 drops of 30% ammonium hydroxide were added and the whole shaken vigorously until froth appeared. The colour of the froth is observed immediately; in a weak reaction it is pink and in a strong one it is a definite red colour.<sup>2</sup> Tests were performed within 24 hours of admission.

**Rapid slide test:** Sclavo diagnostic antigens (Institute Sieroterapico e Vaccinogeno Toscano, Siena, Italy) were used, and, according to the manufacturer's instructions, 1 drop of serum is mixed with 1 drop of either H or O antigen on a slide, and evidence of agglutination looked for after mixing for 1 minute.

## RESULTS

### Typhoid Cases

Table I presents the data on those patients in whom the final diagnosis was typhoid fever. The diagnosis was

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TABLE I. LABORATORY TESTS IN PROVEN TYPHOID CASES

No.	Age	Sex	When first seen after onset	Isolation of <i>S. typhi</i>	Max. Widal titres		Sclavo rapid slide test		Diazo reaction
					O	H	O	H	
1	25	M	1 week	No	320	1 280	++	++	-
2	25	M	1 week	No	160	640	++	++	+
3	26	F	1 week	Blood	320	2 560	++	++	++
4	30	F	2 days	Blood	160	1 280	++	++	+
5	28	M	3 days	Blood	320	2 560	++	++	-
6	45	F	3 days	Blood	160	2 560	++	++	++
7	25	F	1 week	Blood	320	2 560	++	++	++
8	14	M	4 days	No	80	2 560	++	++	+
9	19	F	1 week	Blood	320	640	++	++	-
10	11	M	5 days	No	640	2 560	++	++	ND
11	15	M	1 day	Blood	80	40	++	++	++
				Stool					
12	32	F	2 weeks	Blood	40	80	++	++	-
13	24	F	2 weeks	No	40	320	++	++	-
14	?	M	2 weeks	Blood	160	1 280	++	++	-
15	27	M	2 weeks	Blood	160	2 560	++	++	-
16	29	F	2 weeks	Blood	0	0	++	++	+
				Stool					
17	35	F	2½ weeks	No	320	2 560	++	++	+
18	28	F	4 weeks	Blood	640	2 560	++	++	+
				Stool					
19	35	M	4 weeks	Blood	640	1 280	++	++	-
				Stool					
20	30	M	2 weeks	Blood	160	1 280	++	++	-

++ = strong positive reaction.  
 + = weak positive reaction.  
 - = negative reaction.  
 ND = not done.

based upon isolation of the organism, Widal titres of 160 or greater, and clinical presentations and courses consistent with typhoid fever.

**Non-Typhoid Cases**

Table II presents the final diagnosis in those pyrexial patients admitted during the period of collection of the 20 cases of typhoid.

Of these patients, 5 had positive Widal tests at low titre on screening. The Sclavo test was positive in 4 patients, and the diazo test in 2.

Ten non-typhoid patients in whom the Widal tests were negative had positive diazo tests; 7 were pulmonary infection and 3 malaria. In 4 the reaction was strong.

In the rapid slide test (Sclavo) on the non-typhoid cases with negative Widal tests, 1 was positive for O antigen, and 29 were positive for H antigen. Of these, 20 showed weak to moderate reactions, and 9 strong to very strong ones.

**Phage Typing**

All strains of *Salmonella typhi* isolated in Rhodesia are typed by the laboratory at the University of Pretoria

TABLE II. DIAGNOSIS IN NON-TYPHOID CASES

Final diagnosis	No. of patients
Malaria	18
Pneumonia	15
Pyrexia of unknown origin (undiagnosed)	12
Dysentery (+ other gastro-intestinal infection)	5
Polyarthritits	5
Influenza	4
Meningitis	4
Rheumatic fever	3
Lung abscess	2
Pleurisy	2
Fits, acute nephritis, Ca lung, infectious hepatitis, TB glands, osteomyelitis, URT infection, multiple abscesses, bilharzia, liver abscess	1 each ... 10
<b>Total</b>	<b>80</b>

(Dr C. G. Crocker). The dominant types are A and E1 (75%), but D1, 42 and 21A are also found. The strains isolated in this series were drawn from the first 4 of the types.

## DISCUSSION

A number of practical points emerged from this study.

Isolation of the organism is a fairly successful method of diagnosis no matter what stage of the disease is reached when the patient presents. In 15 patients the organism was isolated after the second week of symptoms. It is noteworthy that the organism can still be isolated from the blood in the presence of high titres of antibody.

In this random sample, however, 30% of cases were negative on one or more attempts at culture of either the blood or stool — which is compatible with figures from other countries.<sup>3-5</sup> It must be accepted that with the high prevalence of other illness in the population, the timing may not be accurate even within fairly wide limits. Such a failure rate, however, clearly indicates the need for other tests.

The Widal test results proved interesting, all but 1 of the typhoid cases being positive. The exception was diagnosed on blood and stool cultures. Anti-H titres attained were higher than anti-O, and comparatively few cases had an anti-H titre at less than 640 or an anti-O titre at more than 320. Titres fluctuated a good deal during admission, and in 1 case reverted to zero a week after admission. In 3 of the 4 cases in which titres failed to rise above 80, organisms were isolated from the blood.

It was noteworthy that even in cases of typhoid presenting during the first week of the disease, high antibody titres were the rule. Indeed the levels suggested an anamnestic type of response rather than a primary one. In the light of the very small number of non-typhoid pyrexial patients who had residual positive Widal tests when screened at 1 in 40, it may be that post-typhoid levels of antibody are considerably lower than this. The absence of detectable antibody may reflect susceptibility, but not necessarily the true immunological status in respect of a particular antigen, a situation known to occur frequently in children<sup>3,4</sup> and possibly in some adults.<sup>6</sup> However, some sensitisation may well have come from related strains of *Salmonella* in which low antibody levels are common. A further possibility is suggested by studies showing that where typhoid and schistosomiasis coexist in the same patient, concomitant antischistosomal treatment is associated with marked clinical improvement in the typhoid condition, and a decrease in the incidence of carriers.<sup>6,7</sup> The effect of schistosomiasis on the Widal reaction is not yet known, but the disease is endemic in Rhodesia and the results of current investigations might prove relevant.<sup>8</sup>

The great variation in Widal titres in individuals in whom typhoid is proved by culture, is confusing. Most surveys indicate that only 90% of patients ever produce detectable anti-H or anti-O antibodies.<sup>9</sup> Our series was taken from consecutive admissions over a period of time when there was no epidemic. The phage types showed a fairly wide distribution and suggested that the different strains were more or less powerful inducers of one or other (or both) type of antibody, though host response has been held to be of greatest importance in the type of response invoked.<sup>10</sup>

Five non-typhoid patients had titres against H antigen or against both H and O antigens of 40 or above. The 4 who had titres of 40 against H, probably had attacks at least a year previously, while 1 had titres compatible, we believe, with a current attack of the disease.

It was concluded with regard to the Widal reaction that the general population in this endemic area has little residual antibody to *S. typhi* at titres of 1 in 40 or more, but the high early response in most cases indicates that the majority of cases had previously encountered *S. typhi* or a related organism. At the time of presentation, be it in the first week or in the eighth, both anti-H and anti-O titres are high, the former reaching greater heights than the latter. In view of this it is proposed that in clinically suggestive cases titres of even 1 in 80 against either antigen indicate probable infection, while titres of even 1 in 160 are virtually diagnostic.

The diazo reaction we did not find helpful. Only 50% of the typhoid group were positive, and even then reactions were largely weak. Of the non-typhoids 10 were also positive, and it must be concluded that the test has no specificity for typhoid. False positives occurred with both malaria and chest infections, a finding which confirmed previous experience.<sup>2</sup>

Rapid slide tests are potentially most useful, particularly where elaborate facilities are not available. However, we were aware that before this study was instituted certain district hospitals had admitted large numbers of cases of typhoid on the basis of these tests, and it was thought that a widespread epidemic had begun. Further investigation revealed that while some people may well have residual antityphoid antibody, positive tests were not synonymous with active typhoid. Positive reactions against the H antigen preparation were shown by 29 Widal-negative cases. The specificity of the O antigen was much greater, there being only one result in disagreement with the Widal tests. Though the tests are intended to become positive only when antibody titres are 1 in 100 or over, positive results were obtained with Widal titres of 1 in 40, a level compatible with past infection or vaccination.

It would seem that some considerable experience may be necessary in the performance and interpretation of these tests in an endemic typhoid area.

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## REFERENCES

1. Wicks, A. C. B., Holmes, G. S. and Davidson, L. A. G. (1971): *Quart. J. Med.*, **40**, 341.
2. Huckstep, R. L. (1962): *Typhoid Fever and Other Infections*. Edinburgh: E. & S. Livingstone.
3. Olitski, A. and Afori, M. (1965): *Israel J. Med. Sci.*, **1**, 607.
4. *Idem* (1966): *Ann. Immunol. (Hungary)*, **9**, 159.
5. Olitski, A. (1972): *Enteric Fever*, pp. 65-66. London: S. Karger.
6. Halawani, A., Abdulla, A. and Badran, A. (1960): *Amer. J. Trop. Med.*, **9**, 371.
7. Neves, J., Marinma, R. P., Martins, N. B., Aravjo, P. K. D. and Lucciola, J. (1969): *Trans. Roy. Soc. Trop. Med. Hyg.*, **63**, 79.
8. Franco, M. and Gelfand, M. (1973): Personal communication.
9. Wilson, G. S. and Miles, A. A. in Topley, W. C. and Wilson, G. S., eds (1964): *Principles of Bacteriology and Immunity*, 5th ed. Baltimore: Williams & Wilkins.
10. Felix, A. (1924): *J. Immunol.*, **9**, 111.