

Serological response of patients infected with *Salmonella typhi*

H Chart, B Rowe, J S Cheesbrough

Abstract

Aims—To evaluate a rapid immunoblotting procedure for providing evidence of infection with *Salmonella typhi* using 73 sera from patients infected with *S typhi*.

Methods—A sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)/immunoblotting procedure using lipopolysaccharide (LPS, O=9,12) and flagellar (H=d) antigens was used.

Results—Seventy two of 73 sera contained antibodies to LPS, 40 sera also contained antibodies to H=d flagellar antigens. Analysis of acute and convalescent sera showed that only 62% of patients produced antibodies to flagellar antigens.

Conclusions—The SDS-PAGE/immunoblotting procedure provided a rapid method for providing serological evidence of infection with *S typhi*.

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Typhoid fever continues to be a major public health problem in developing countries, and although its incidence is lower in northern Europe and North America, cases of typhoid are found frequently as a result of travellers returning from endemic areas. The diagnosis can be confirmed by blood culture in the majority of patients who have not received the appropriate antibiotics. Although cultures of stool, urine, and bone marrow yield some additional isolates, in a small proportion of cases all cultures may be negative for *Salmonella typhi* and the diagnosis then rests on a combination of clinical and epidemiological features. In such cases, serological methods offer an alternative approach and, therefore, retain an important place in the diagnosis of typhoid.

The antigenic structure of *S typhi* is (Vi) 9, 12:d¹; and infection with *S typhi* may result in the production of serum antibodies to the O=9,12 somatic, the H=d flagellar, and the "Vi" capsular antigens. For many years the Widal agglutination test has been used to detect antibodies to these antigens²; however, more modern immunoassays have been considered as a replacement.

In a previous study, the techniques of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were used as a comparison with the established Widal technique for the examination of sera for antibodies to the lipopolysaccharide (LPS) and flagellar antigens of *S typhi*.³

In the present study, sera from patients who were all culture positive for *S typhi* were used to evaluate this rapid form of serodiagnosis more fully.

Methods

BACTERIA

Salmonella enteritidis strain P132344 (O=1,9,12; H=g,m) was used for the preparation of LPS and *S meunchen* strain JT54 (O=6,8; H=d) was used to prepare flagella.³ Bacteria were grown on nutrient agar (37°C for 16 hours) from Dorset's egg agar slopes stored in the Laboratory of Enteric Pathogens, London.

PATIENTS

The 47 patients (33 male, 14 female) involved in the study had typhoid fever and were aged between 18 and 50 years. All were hospitalised in the Infectious Disease Hospital, Kuwait. They were expatriate workers from Kuwait (3), Jordan (3), Syria (2), Iraq (1), India (11), Bangladesh (6), Pakistan (13), Sri Lanka (2), unknown (6). Patients were all culture positive for either blood or faecal *S typhi*.

SERA

Seventy three serum samples were obtained from the 47 patients. Both acute and convalescent sera were obtained from 26 patients, and either an acute or a convalescent serum was obtained for 21 patients. Twelve sera were from healthy controls from the United Kingdom, their vaccination details were not known. All sera were stored at -30°C until used.

LIPOPOLYSACCHARIDE

Lipopolysaccharide was prepared from *S enteritidis* strain P132344, using proteinase K (Sigma, Poole, Dorset, UK) and hot phenol extraction as described previously.³ Outer membranes prepared from *S enteritidis* formed the basis for hot phenol extraction using the method of Westphal and Jann.⁴

FLAGELLA

Flagella were extracted from *S meunchen* strain JT54.² Bacteria harvested from 6 × 15 cm nutrient agar plates were suspended in saline and the preparation was incubated at 60°C for 30 minutes. Bacteria were sedimented by centrifugation (12 500 ×g for 10 minutes) and the supernatant containing extracted flagella was removed and stored at -30°C. The protein concentration of flagellar preparations was determined using the method of Lowry *et al*,⁵ and the *S meunchen* flagellar subunit size was

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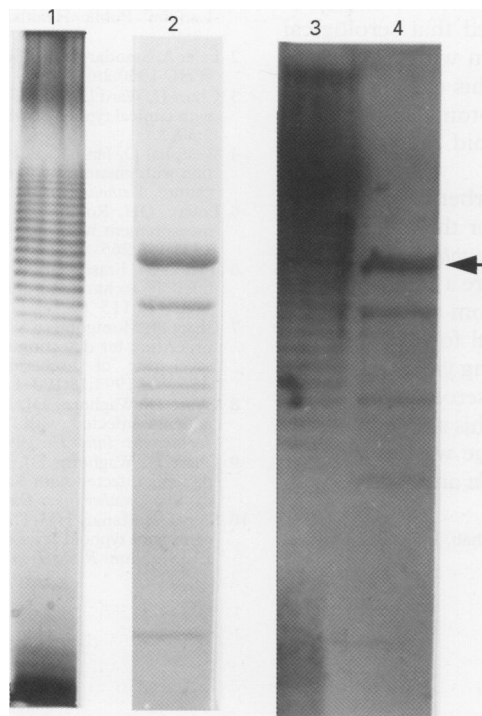


Figure 1 Seventy two sera contained antibodies to the lipopolysaccharide (LPS) of *S enteritidis* (lane 1), giving a strong reaction by immunoblot (lane 2). Forty sera also contained antibodies to the 65 kDa flagellar subunits of *S meunchen* (lane 3), as shown by the immunoblot arrowed in lane 4.

Table 1 Analysis of 73 serum samples from 47 patients culture-positive for antibodies to antigens O=9,12 and H=d

Sera	Antibodies to	
	O=9,12	H=d
Acute sera (n=26)*	25	16
Convalescent sera (n=26)	26	18
Acute only (n=16)	16	11
Convalescent only (n=5)	5	3

*Number of patients.

determined by performing SDS-PAGE alongside a commercial preparation of protein molecular weight standards (Bio-Rad, Hemel Hempstead, UK).

SDS-PAGE/IMMUNOBLOTTING

SDS-PAGE and immunoblotting were performed² using an Atto mini gel system (Genetic Research Instruments, Dunmow, Essex, UK). For SDS-PAGE, 1 µg of LPS or 10 µg flagellar protein was loaded per lane on to a gel comprising a 4.5% stacking gel and a 12.5% separation gel. Following electrophoresis (50 mAmps for 30 minutes), resultant profiles were either stained for LPS using a silver stain for carbohydrate⁶ or used for immunoblotting and reaction with patients' antibodies. For immunoblotting, profiles were transferred on to nitrocellulose membranes (0.5 Amps for 1.5 hours), blocked with skimmed milk (3% dried milk powder in phosphate buffered saline (PBS)), and reacted with sera (30 µl per lane). Immunoblots were then washed (three times) with PBS containing 0.05% Tween 20, and bound antibodies detected with a goat antihuman polyvalent antibody conjugated with alkaline phosphatase

(Sigma).² Antibody-antibody conjugate complexes were detected with an enzyme substrate comprising 20 ml of 0.1 M Tris, 0.09 M NaCl, and 0.15 M Mg₂Cl₂·6H₂O containing 90 µl nitroblue tetrazolium (Sigma; 75 mg/ml in 70% aqueous dimethyl formamide) and 70 µl of aqueous 5-bromo-4-chloro-3-indolylphosphate (Sigma; 50 mg/ml).⁷⁻⁹

Results

CONTROL SERA

The 12 control sera did not contain antibodies to the O=9,12 LPS, or H=d flagellar antigens.

PATIENTS' SERA

Seventy two of 73 patients' sera contained antibodies binding to SDS-PAGE profiles of O=1,9,12 LPS (fig 1, lane 1); fig 1 (lane 3) gives an example of a typical LPS immunoblot result. Forty sera also contained antibodies to the H=d flagellar antigen, migrating as a band of 65 kDa (fig 1, lane 2); fig 1 (lane 4) gives an example of a typical flagellar immunoblot result. The serum without antibodies to LPS contained antibodies to H=d flagellar antigens.

The panel included acute and convalescent sera from 26 patients (table 1), 25 of the 26 acute sera contained antibodies to the O=9,12 antigens while all of the 26 convalescent sera contained antibodies to these antigens (table 1). Sixteen of the 26 acute sera and 18 of the 26 convalescent sera contained antibodies to the H=d antigen (table 1). Eight patients did not have anti-flagellar antibodies in either acute or convalescent sera and two patients had anti-flagellar antibodies in convalescent sera only (table 2).

Discussion

In the present study we used 73 sera from culture positive patients with typhoid to evaluate an immunoblotting procedure based on the O=9,12 LPS antigens and H=d flagellar antigens. Because *S enteritidis* and *S typhi* express the same O=9,12 antigens, *S enteritidis* was used to prepare LPS as a safety precaution. On its own, the presence of serum antibodies to the O=9,12 LPS antigens is not a reliable indicator of infection with *S typhi*, because patients infected with *S enteritidis* have also been shown to produce antibodies to the O=9,12 antigen.^{8,9} However, the detection of serum antibodies to flagellar antigen H=d, reinforces the diagnosis. Although other serotypes of salmonella possess the O=9,12 and H=d antigens, these bacteria are isolated only very rarely⁹ and antibodies to the O=9,12 and H=d antigens would be highly indicative of infection with *S typhi* in the UK.

Acute and convalescent sera were obtained from 26 patients. Of these, antibodies to the H=d antigen could not be detected in either acute or convalescent sera from eight of these patients. Whether this was due to the lack of flagellar antigen expression during infection, or the inability of patients to produce antibodies to flagellar antigens, was unknown. However, this result showed that approximately one third of patients did not produce antibodies to these flagellar antigens, and that the inability to detect patients' antibodies to H=d flagellar,

Table 2 Analysis of 52 serum samples from 26 patients culture-positive for antibodies to antigen H=d

	Number positive
Acute and convalescent	16
Neither acute nor convalescent	8
Acute but not convalescent	0
Convalescent but not acute	2

should not rule out a possible infection with *S typhi*. It should be recognised that serological tests for evidence of infection with *S typhi* are not as reliable as culturing this organism from a patient, and patients' symptoms and any history of vaccination for typhoid must be taken into consideration.

Enzyme linked immunosorbent assays (ELISAs) have been described for the detection of antibodies to the LPS of *S typhi*.³⁻¹⁰ Although such quantitative assays require a cut off value to delineate antibody positive from antibody negative sera, they are very useful for obtaining an antibody titre. Immunoblotting yields an easy to read qualitative result for the serology of patients with suspected typhoid, and this study illustrates the potential of the technique we describe for detecting antibodies to *S typhi* antigens.

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