Epidemiol. Infect. (1990), **105**, 79–85
Printed in Great Britain

Serological grouping of *Treponema hyodysenteriae*

D. J. HAMPSON, J. R. L. MHOMA, B. G. COMBS AND J. I. LEE
School of Veterinary Studies, Murdoch University, Perth, Western Australia 6150

(*Accepted 14 February 1990*)

SUMMARY

Two Australian isolates of *Treponema hyodysenteriae* which did not fit within the current serological grouping system for these bacteria were examined by agarose gel double immunodiffusion tests (AGDP). Isolate Vic1 was serologically unique, and we propose that it becomes the type organism for a new sixth serological group of *T. hyodysenteriae* (Group F). Isolate Q1 was unusual in that lipopolysaccharide (LPS) extracted from it reacted strongly in AGDP with serum raised against the type organism for serogroup D (A1), and also weakly with serum raised against the type organism for serogroup B (WA1). The nature of this cross-reactivity was examined by using cross-absorbed antisera in AGDP, and by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blot analysis.

The pattern of serological cross-reactivity between Q1, A1 and WA1 was complex and was not fully defined, but the isolate Q1 apparently shared low molecular weight 'serogroup' LPS antigens with A1, and shared higher molecular weight LPS antigens with WA1. On this basis Q1 was designated as belonging to serogroup D, although it was recommended that this be qualified as D (B) to indicate the presence of weak cross-reactivity with serogroup B. Such serological cross-reactivity may have significance in relation to the development of immunity to *T. hyodysenteriae*. Isolate Q1 may be a potentially useful organism for vaccine development because of its ability to induce a good serological response to LPS of treponemes from both serogroups D and B.

INTRODUCTION

Treponema hyodysenteriae is an enteric spirochaete which is the aetiological agent of swine dysentery [1, 2]. This bacterium can be typed serologically by agarose gel double diffusion precipitation tests (AGDP) using extracted lipopolysaccharide (LPS) and hyperimmune rabbit antiserum raised against whole organisms [3]. The microorganisms were initially divided into four serotypes [3], with at least three more serotypes later described [4, 5]. More recently it has been proposed that the organisms be arranged into five serogroups [6], with members of each group being defined by the reactivity of their LPS with rabbit serum raised against a 'type' organism for the serogroup. Serogroup antigens apparently consist of three or four major antigenic components in the 10–42 kDa range [7]. Within a serogroup, isolates which possess additional unique LPS antigens which can be identified using cross-absorbed sera can be regarded as serotypes or serovars within the serogroup [6].

During a study of the epidemiology of *T. hyodysenteriae* in Australia, we have encountered isolates which do not fall within the current serological grouping system. The purpose of the present paper is to describe these isolates and to suggest modifications to and an enlargement of the serological typing system now in use.

MATERIALS AND METHODS

The seven treponemes used in the study are listed in Table 1. These included type organisms B78, WA1, A1 and WA6 as previously described [6], B169 which was originally isolated in Canada [3] and which was provided by Dr T. B. Stanton, the National Animal Disease Centre, Ames, Iowa, USA, isolate Q1 which was received from R. Thomas, Animal Research Institute, Yeerongpilly, Queensland, Australia and isolate Vic1 which was provided through Dr R. T. Jones, Regional Veterinary Laboratory, Bendigo, Victoria, Australia. All microorganisms were grown in Trypticase Soy Broth, and used for LPS extraction, production of antisera and absorption of selected antisera as previously described [6]. The sera which were absorbed and the treponemes used in the process are indicated in Table 2.

Lipopolysaccharide extracted from the bacteria and unabsorbed and selected absorbed antisera were reacted against each other in AGDP at least twice. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting of LPS from treponemes WA1, A1 and Q1 were conducted as previously described [7], using both unabsorbed antisera raised against these three isolates and sera which were cross-absorbed with each of the three isolates.

RESULTS

Results of AGDP using unabsorbed antisera are presented in Table 1. Lipopolysaccharide from type organisms for serogroups A to E all reacted with rabbit antiserum raised against themselves. Lipopolysaccharide from Vic1 reacted only with antiserum raised against itself, and this antiserum did not react with LPS from any of the other treponemes tested. Lipopolysaccharide from Q1 reacted strongly with antisera raised against both A1 and Q1, and weakly with antiserum raised against WA1. Antiserum raised against Q1 reacted strongly with LPS from A1, Q1 and WA1.

Results of AGDP with LPS from A1, WA1 and Q1 and unabsorbed antisera and antisera cross-absorbed with the three isolates are presented in Table 2. In all cases absorption of antiserum with the homologous organism removed all activity in AGDP. Cross-absorption of antiserum against A1 removed all its activity against LPS from both A1 and Q1 when either of these two bacteria were used to absorb the antiserum, but absorption with WA1 cells did not influence the serum's reactivity. Cross-absorbing antiserum against WA1 with A1 or Q1 cells removed its weak activity against Q1 but did not remove its activity against WA1. The activity of antiserum against Q1 with LPS from WA1 was removed by absorbing with WA1 cells, and its activity against A1 was similarly removed by absorbing with A1 cells.

Results of SDS–PAGE and Western blotting with LPS from the above isolates

Table 1. *Agarose gel double immunodiffusion between lipopolysaccharide extracts of* Treponema hyodysenteriae *and rabbit antisera*

Isolate (LPS)	LPS serogroup*	Antisera against B78	WA1	B169	A1	WA6	VIC1	Q1
B78	A	+						
WA1	B		+					+
B169	C			+				
A1	D				+			+
WA6	E					+		
VIC1	NT						+	
Q1	NT		±		+			+

* Hampson et al. [6]. NT, Not typed; ±, reaction present but weak.

Table 2. *Agarose gel double immunodiffusion between lipopolysaccharides of* Treponema hyodysenteriae *and cross-absorbed rabbit antisera*

Absorbing organism	Antiserum against	Bacterial isolates (LPS) A1	WA1	Q1
—	A1	+	−	+
WA1	A1	+	−	+
A1	A1	−	−	−
Q1	A1	−	−	−
—	WA1	−	+	±
WA1	WA1	−	−	−
A1	WA1	−	+	−
Q1	WA1	−	+	−
—	Q1	+	+	+
WA1	Q1	+	−	+
A1	Q1	−	+	+
Q1	Q1	−	−	−

± Reaction present but weak.

and antisera are shown in Fig. 1. Antiserum raised against A1 (membrane a) reacted with its own LPS components (lane 1), especially with a broad band of reactivity around 19 kDa and another around 27 kDa, with at least another nine sharper bands of greater molecular weight ranging from around 35 to 60 kDa. This antiserum also reacted with similar bands in the LPS preparation from Q1 (lane 2). but only with a few high molecular weight bands of LPS from WA1 (lane 3). Antiserum raised against WA1 (membrane b) only reacted faintly with a few high molecular weight bands of LPS from A1, reacted strongly with all the major bands of Q1 which had reacted with antiserum against A1, and reacted strongly with LPS components from WA1. In particular against WA1 it reacted with broad heavy bands of molecular weights around 38, 23 and 15 kDa, with smudging between bands. Antiserum raised against Q1 (membrane c) reacted with all the major components of A1, Q1 and WA1 detected by the two previous sera, except that its reaction with LPS components of A1 was weaker than those seen using

https://doi.org/10.1017/S0950268800047671 Published online by Cambridge University Press

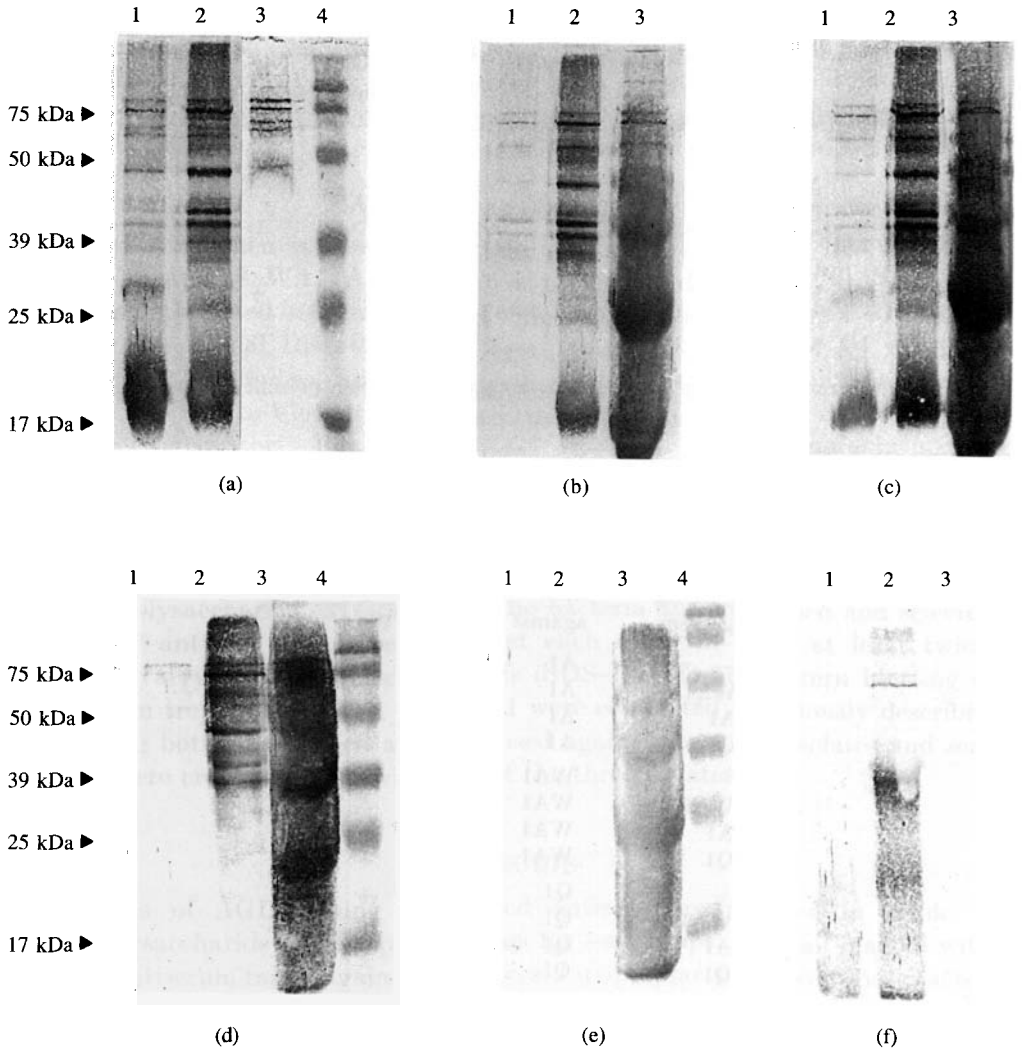

Fig. 1. Immunoblots of *T. hyodysenteriae* LPS. For each membrane, lane 1 contains A1, lane 2 Q1, lane 3 WA1 and lane 4 where shown contains molecular weight markers. Membranes (a), (b), (c), (d), (e) and (f) reacted with antisera against A1, WA1, Q1, Q1 absorbed with A1 cells, WA1 absorbed with Q1 cells, and Q1 absorbed with WA1 cells respectively.

serum raised against A1. Absorption of serum against A1 with Q1 cells removed all activity against LPS from both bacteria (membrane not shown). Absorption of serum against Q1 with A1 cells (membrane d) removed all activity against A1 LPS, and also that against the two main low molecular weight bands in Q1, but all other reactivity was retained. Absorption of serum against WA1 with either Q1 (membrane e) or A1 cells (membrane not shown) removed all activity against LPS from both A1 and Q1, but reactivity against all components of WA1 LPS was retained. When the serum against Q1 was absorbed with WA1 cells (membrane f), activity against the low molecular weight LPS components of A1 and Q1 was

https://doi.org/10.1017/S0950268800047671 Published online by Cambridge University Press

retained (although reduced, particularly in the case of A1), but all other reactivity was removed.

DISCUSSION

Lipopolysaccharide from Victorian treponemal isolate Vic1 would not react in AGDP with antisera raised against the five type organisms for *T. hyodysenteriae* serogroups A to E. Antiserum raised in a rabbit against formalized Vic1 cells would also not react with LPS from any of the five type organisms, although it did react with LPS from Vic1. Vic1 was confirmed as being *T. hyodysenteriae* on the basis of its strong beta-haemolysis, production of indole, API ZYM profile [8] and Multilocus Enzyme Electrophoresis grouping [9]. We therefore propose that Vic1 be regarded as a type strain for a new sixth serological group of *T. hyodysenteriae* (group F). We have found no other treponemes belonging to this serogroup to date.

The Queensland isolate Q1 behaved unusually in that LPS extracted from it reacted in AGDP with antiserum raised against A1 (the type organism for serogroup D), but also weakly with antiserum raised against WA1 (the type organism for serogroup B). On this basis Q1 would appear to span serogroups D and B. When antiserum was raised against Q1 it reacted strongly with LPS from Q1, A1 and WA1 even though antiserum against A1 did not react with LPS from WA1 and *vica versa*. These cross-reactivities were confirmed by Western blotting, the only differences being that antiserum against WA1 reacted strongly with all the major components of Q1 LPS, and antiserum against Q1 reacted more weakly against A1 LPS components than it did against its own LPS components. As in a previous study [7], some higher molecular weight bands were found to be shared by organisms from different serogroups.

This unusual pattern of serological reactivity seen with Q1 was not due to contamination of the Q1 LPS preparation with LPS from another treponeme, since the same Q1 banding patterns were observed with all three antisera used in Western blot analysis. Absorption of the serum against Q1 provided a possible partial explanation of the reactivities seen. Thus when the serum was absorbed with A1 cells it would no longer react with low molecular weight LPS bands in Q1, although higher molecular weight material was still stained (membrane d). Previously these missing lower molecular weight LPS bands of *T. hyodysenteriae* (in the range 10–42 kDa) have been equated with serogroup antigens [7]. In contrast when this same antiserum was absorbed with WA1 cells, the lower molecular weight bands in Q1 were still stained (as were those in A1, but much more weakly) whilst the higher molecular weight material was no longer visible (membrane f). It would therefore appear that Q1 shares low molecular weight (serogroup) antigens with A1 (the type organism for serogroup D), and shares higher molecular weight material with WA1 (the type organism for serogroup B). This simple explanation does not however indicate why unabsorbed antiserum against Q1 reacted with all the major LPS components of both A1 and WA1 (membrane c), why antisera against either A1 or WA1 reacted with all the LPS components of Q1 (membranes a and b respectively), or why absorption of serum against WA1 with either A1 or Q1 cells removed all activity against LPS components of Q1 (membrane e) whilst absorption of serum against A1 with WA1 cells had no effect on its reactivity with Q1 LPS (membrane not shown).

These unpredictable and complex antigenic relationships present problems for the serological typing system in use for *T. hyodysenteriae*. In the case of isolate Q1, it seems justified to call it serogroup D on the basis of a strong reaction between its LPS and antiserum against A1, particularly as this reactivity appeared to be directed against low molecular weight 'serogroup' antigens. The weak reactivity with serum raised against WA1 should either be ignored, or acknowledged by qualifying its serogroup as D(B). Mapother and Joens [4], when they encountered similar weak cross-reactivities between 'serotypes' of *T. hyodysenteriae*, recommended that only strong AGDP reactions developing within 6 h be considered positive. The problem with this approach is that it fails to give additional important information about the serological properties of an isolate. In the case of isolate Q1, a bacterin prepared from it can be used to give a good immunological response to LPS from organisms of both serogroups D and B (and for that reason may be a useful strain to use for vaccine production), even though the isolate is apparently of serogroup D.

It is likely that further difficulties will arise with the serogrouping system for *T. hyodysenteriae*. The apparent strength of serological reactivities between specific antisera and LPS from various treponemal isolates varies considerably, and homologous antiserum raised against a given isolate usually gives a better reactivity with LPS from the isolate than does specific typing serum. This is presumably due to small differences in antigenic composition of the LPS between isolates, and makes interpretation of AGDP results difficult if the strength of a serological reactivity is an important criterion in assigning an isolate to a serogroup. It seems likely that in the future isolates will be found which clearly span one or more serogroups as currently defined, and when that occurs either the serogrouping system will need to be redefined or the organisms assigned to more than one serogroup.

Some LPs components from the isolates examined by SDS–PAGE and Western blotting in this study had slightly different molecular weights from those previously recorded by us [7]. For example an LPS band in A1 previously recorded at 12 kDa was recorded here at 19 kDa, and bands in WA1 at 42 and 30 kDa were located at 38 and 23 kDa respectively in the present study. These differences are probably attributable to the use of short non-preparative polyacrylamide gels (6 cm) in both studies, together with possible minor differences in sample preparation or storage.

ACKNOWLEDGEMENTS

This work was supported by grants from the Australian Pig Research Council, the Murdoch University Special Research Fund and the Western Australian Pig Industry Compensation Fund.

REFERENCES

1. Taylor DJ, Alexander TJL. The production of dysentery in swine by feeding cultures containing a spirochaete. Br Vet J 1971; **127**: 58–61.
2. Harris DL, Glock RD, Christensen CR, Kinyon JM. Swine dysentery. Inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. Vet Med Small Animal Clinician 1972; **67**: 61–4.
3. Baum DH, Joens LA. Serotypes of beta haemolytic *Treponema hyodysenteriae*. Infect Immun 1979; **25**: 792–7.

4. Mapother ME, Joens LA. New serotypes of *Treponema hyodysenteriae*. J Clin Microbiol 1985; **22**: 161–4.
5. Lemcke RM, Bew J. Antigenic differences among isolates of *Treponema hyodysenteriae*. Proc Int Pig Vet Soc Cong, Ghent 1984; 183.
6. Hampson DJ, Mhoma JRL, Combs BG, Buddle JR. Proposed revisions to the serological typing system for *Treponema hyodysenteriae*. Epidemiol Infect 1989; **102**: 75–84.
7. Hampson DJ, Mhoma JRL, Combs BG. Analysis of lipopolysaccharide antigens of *Treponema hyodysenteriae*. Epidemiol Infect 1989; **103**: 275–84.
8. Hunter D, Wood T. An evaluation of the API ZYM system as a means of classifying spirochaetes associated with swine dysentery Vet Rec 1979; **104**: 383–4.
9. Lymbery AJ, Hampson DJ, Hopkins RM, Combs M, Mhoma JRL. Multilocus enzyme electrophoresis for identification and typing of *Treponema hyodysenteriae* and related spirochaetes. Vet Micro 1990, In press.