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Slide-agglutination for rapid serological typing of *Treponema hyodysenteriae*

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SUMMARY

A slide agglutination (SA) test was developed to determine the serogroup of isolates of *Treponema hyodysenteriae* of serogroups A to F. Rabbit antisera which are normally used for serogrouping *T. hyodysenteriae* in an agarose gel double-diffusion precipitation test (AGDP) were not suitable for SA because they agglutinated isolates from more than one serogroup. The agglutination reaction was made serogroup-specific by cross-absorbing the typing sera for serogroups A to F with whole treponemes from the other 5 of these 6 serogroups of *T. hyodysenteriae*. The absorbed sera were reacted in slide agglutination tests with 33 isolates of *T. hyodysenteriae* and with four non-*T. hyodysenteriae* intestinal spirochaetes. None of the non-*T. hyodysenteriae* isolates agglutinated, but 27 of the 33 isolates of *T. hyodysenteriae* did. The results for 26 of the 27 agglutination reactions agreed with the serogroup as determined in AGDP. One of the 6 isolates of *T. hyodysenteriae* which failed to react in slide agglutination was of serogroup B, 1 of serogroup D, 1 each were from new serogroups G, H and I, and 1 was untypable in AGDP.

INTRODUCTION

Swine dysentery is a mucohaemorrhagic colitis of pigs resulting from infection with the anaerobic spirochaete *Treponema hyodysenteriae* [1, 2]. These bacteria can be typed for epidemiological studies using serological methods [3, 4]. This procedure involves extracting lipopolysaccharide (LPS) from the isolate to be typed, and reacting this in agarose gel double diffusion precipitation tests (AGDP) with hyperimmune rabbit antisera raised against type strains of the main serogroups of *T. hyodysenteriae* [4]. To date nine serological groups of the bacteria have been described [4–6]. Immunity against *T. hyodysenteriae* infection in colonic loops is LPS-serotype specific [7], and bacterin vaccines confer the best protection when subsequent infection is with an homologous LPS-serotype of the bacteria [8]. Serological typing therefore provides valuable practical information about any given isolate.

Currently the major drawback of serological typing of *T. hyodysenteriae* is the necessity to grow the isolate in large quantities, and to extract LPS for use in AGDP. The purpose of the present study was to attempt to develop a rapid and

simple serological typing method which would be suitable for routine use in veterinary laboratories.

MATERIALS AND METHODS

Microorganisms

The treponemal isolates used in the study and their origin are indicated in Table 2. *Treponema hyodysenteriae* strains B78, WA1, B169, A1, WA6, Vic1, Q16, Vic2 and NSW1 are type strains for serogroups A to I respectively [4–6]. The other 24 *T. hyodysenteriae* isolates used in the study were confirmed as such on the basis of their strong beta-haemolysis on 5% defibrinated sheep blood agar, production of indole, and characteristic biochemical profile in the API–ZYM test [9]. Four isolates of non-*T. hyodysenteriae* intestinal spirochaetes from pigs were also used in the study: these were the type strain of *Treponema innocens* (B256), two UK isolates from this same 'species' (PWS/A, 4/71), and a porcine intestinal spirochaete (P43/6) recovered from 'spirochaetal diarrhoea' [10]. The 33 *T. hyodysenteriae* isolates were serogrouped in AGDP as previously described, using rabbit antisera prepared against the 'type strains' of the nine serogroups [4–6]. Only the 'type strains' of serogroups C, F, G, H and I were available for use in the study. One of the isolates examined was untypable in AGDP with currently available antisera.

Preparation of antisera

Rabbit antisera were prepared against *T. hyodysenteriae* 'type strains' for serogroups A to F as previously described [4, 5]. Antiserum was also raised against the LPS of *T. hyodysenteriae* strain WA1. The LPS was first extracted and quantified on the basis of hexose content as previously described for use in AGDP [3]. Preparations containing 50 μg of hexoses were then suspended in 1 ml of Freund's complete adjuvant and injected intramuscularly into a rabbit twice at a 3-week interval; this procedure was followed by a series of five weekly intravenous injections with doubling hexose concentrations starting at 10 μg in the first injection and increasing to 160 μg at the fifth. The rabbit was bled 1 week after the last injection.

Absorption of antisera

Each of the 6 antisera raised against the 'type strains' of *T. hyodysenteriae* for serogroups A to F were cross-absorbed with the other 5 'type strains'. The bacterial cells used for absorption were grown, as previously described [4], in the prereduced anaerobic sterilized liquid medium of Kunkle, Harris and Kinyon [11]. The bacteria were pelleted by centrifugation from 350 ml batches of broth, resuspended and washed twice in phosphate-buffered saline (PBS; pH 7·2). Six pooled batches of bacterial cells were then prepared, each 'pool' comprising an equal volume of wet pelleted cells from different combinations of 5 of the 6 serogroups A to F. Cells from each of these six pools were then thoroughly mixed by pipette with an equal volume of serum raised against the serogroup not represented in that cell pool. Each serum-cell suspension was incubated at 37 °C for 30 min, and then left overnight at 4 °C on a rotary shaker. The absorbing

Table 1. *Slide agglutination of 'type strains' of* Treponema hyodysenteriae *for serogroups A to F using rabbit antiserum either raised against the six 'type strains' or against lipopolysaccharide extracted from WA1*

Antiserum raised against	Serogroup	*T. hyodysenteriae* cells					
		B78	WA1	B169	A1	WA6	Vic1
B78	A	+++	++	++	++	+	−
WA1	B	+	+++	−	−	++	−
B169	C	++	++	+++	−	−	−
A1	D	+	+	+	++	−	+
WA6	E	+	+	−	−	+++	−
Vic1	F	+	−	+	−	−	+++
LPS of WA1	B	+++	+++	+++	++	+++	+++

+, Fine agglutination developing after 30 sec; ++, more rapidly developing and coarser agglutination; +++, rapidly developing coarse agglutination

bacteria were then removed from the sera by two 10 min cycles of centrifugation at 15000 *g*.

Slide agglutination (SA)

The spirochaetes to be tested were grown for 3 days on trypticase soy agar plates (BBL) supplemented with 5% defibrinated bovine blood, under an atmosphere of 94% hydrogen and 6% carbon dioxide at 37 °C. Surface growth was harvested from the plates using sterile glass microscope slides, and the bacteria were resuspended in PBS to an optical density of approximately 1·0 at a wavelength of 550 mm. The bacterial suspensions were coded and tested by slide agglutination: the 'type strains' were first tested against the unabsorbed sera, and against the serum raised against the LPS of WA1, and then all the spirochaetal isolates were tested with the six absorbed sera. Single drops of the bacterial suspensions were placed on alcohol-cleaned microscope slides and equal volumes of serum or PBS were added. Each slide was rocked gently for 30 s and then examined for bacterial agglutination against a bright incidence light. Where agglutination did not occur after 30 s with any of the six typing sera, the slides were continued to be observed for up to 2 min. For each serum, results were recorded as either negative or positive for agglutination, with +++ being recorded for rapidly developing coarse agglutination, ++ for slower and less coarse agglutination, and + for fine agglutination only occurring after 30 s.

RESULTS

The results of SA using unabsorbed typing sera against serogroups A to F, and serum raised against the LPS of WA1 are presented in Table 1. The range of strains agglutinating with these sera was variable, as was the degree and rapidity of development of agglutination. All sera however reacted strongly with the strain used to raise the serum, as well as with others. The serum raised against LPS extracted from WA1 agglutinated the type strains of the six serogroups A to F, and also reacted in AGDP with LPS extracted from these bacteria.

The results of serogrouping the *T. hyodysenteriae* isolates by the SA technique

Table 2. *Isolates of* Treponema hyodysenteriae *serogrouped by agarose gel diffusion precipitation (AGDP) and slide agglutination using cross-absorbed antisera*

Isolate	Origin	Serogroup		Nature of agglutination
		AGDP	SA	
B78	USA	A	A	+++
WA15	WA	A	A	+++
WA27	WA	A	A	++
Vic27	Vic	A	A	+
Vic28	Vic	A	A	++
Vic29	Vic	A	A	+
SA3	SA	A	A	++
WA1	WA	B	B	+++
WA26	WA	B	B	+++
B204	USA	B	B	++
Vic5	Vic	B	B	+++
Vic30	Vic	B	B	+++
Vic31	Vic	B	B	++
Vic32	Vic	B	B	++
Vic34	Vic	B	NT	−
B169	Can	C	C	+++
A1	UK	D	D	++
Q2	Queens	D	D	++
Q3	Queens	D	D	+
Q17	Queens	D	NT	−
NSW2	NSW	D	D	++
Vic21	Vic	D	D	+
Vic23	Vic	D	E	++
WA6	WA	E	E	+++
WA3	WA	E	E	++
WA5	WA	E	E	++
KF9	UK	E	E	++
MC52/90	UK	E	E	+++
Vic1	Vic	F	F	++
Q16	Queens	G	NT	−
Vic2	Vic	H	NT	−
NSW1	NSW	I	NT	−
Vic33	Vic	NT	NT	−

NT, not typed; WA, Western Australia; Vic, Victoria; Can, Canada; Queens, Queensland; NSW, New South Wales; SA, South Australia.

+, Fine agglutination developing after 30 s; ++, more rapidly developing and coarser agglutination; +++, rapidly developing coarse agglutination.

using absorbed sera are presented in Table 2, together with a comparison of results of serogrouping by AGDP. None of the *T. hyodysenteriae* isolates autoagglutinated in PBS, and none of the four non-*T. hyodysenteriae* isolates agglutinated with the absorbed sera. There was good agreement on results of serogrouping by AGDP and by SA using cross-absorbed antisera. Of the 29 *T. hyodysenteriae* placed into groups A to F by AGDP, 26 (89·6%) were similarly identified in SA. One isolate of group B (Vic34) and one of group D (Q17) did not agglutinate. The only isolate to be identified differently by the two tests was Vic23, which was placed into serogroup D by AGDP, but which was identified as being of serogroup E using the SA test. Isolate Vic33 was not typed by either technique.

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DISCUSSION

A rapid slide agglutination test has previously been used for the identification of *T. hyodysenteriae* [12]. In that test antiserum raised against *T. hyodysenteriae* isolate P18A (serogroup D) was absorbed with non-*T. hyodysenteriae* isolates PWS/A and 4/71 to remove its cross-reactivity against the non-*T. hyodysenteriae* intestinal spirochaetes. Isolate P18A is a derivative of A1 (R. J. Lysons, personal communication), so it was of interest to see in the current work that of the six unabsorbed sera against the 'type strains' for serogroups A to F, it was only serum raised against A1 that agglutinated all the *T. hyodysenteriae* isolates of these six serogroups (Table 1). Antiserum raised against isolates from any one of the other serogroups would apparently not agglutinate all *T. hyodysenteriae* isolates.

Serogrouping of *T. hyodysenteriae* is based on reacting LPS extracted from the isolate to be typed with antisera raised against 'type strains' for the serogroups. This process is slow, cumbersome and expensive. It seemed possible that an alternative method could be developed by immunizing rabbits with LPS extracted from the 'type strains', rather than the whole bacteria, so that a specific antiserum could be made that could be used to interact directly with an isolate that was to be typed. Unfortunately antiserum raised against the LPS of WA1 strongly agglutinated all the six 'type strains' used in the SA test. This cross-reactivity was presumably either the result of shared antigenic components on the LPS of isolates from the six serogroups, or due to the presence of contaminant cross-reacting protein components in the LPS preparation of WA1. Whatever the cause of this cross-reactivity, it prevented the use of immunizing with crude LPS extracts as a means of obtaining specific typing sera for use in SA.

The alternative approach used to prepare the desired serum was to cross-absorb each of the six sera against the 'type-strains' of serogroups A to F with whole bacterial cells from the 'type-strains' of the other five of these serogroups. This procedure should theoretically remove all reactivity against any shared treponemal components from the serum, leaving activity against serogroup-specific LPS components of the desired strain. This absorption strategy did work, leaving each serum relatively specific for a serogroup of *T. hyodysenteriae* in the SA test, and removing cross-reactivity against non-*T. hyodysenteriae* intestinal spirochaetes. The degree and rapidity of development of agglutination with a given serum varied between isolates, presumably due to differences in the amount or exposure of surface LPS molecules on the various isolates, or due to differences in sample preparation. Only one *T. hyodysenteriae* isolate (Vic23) gave different reactions in the AGDP and SA tests. This isolate was recorded as serogroup E by the SA test on two occasions, but in AGDP it reacted weakly with the group D serum, and not at all with the group E serum. This discrepancy was presumably due to differences in configuration or accessibility of LPS components present in LPS extracts as compared to their arrangement in whole bacteria. This different reactivity need not be a major problem when samples are being typed, so long as the laboratory undertaking the serological typing specifies the method used.

Six *T. hyodysenteriae* isolates were not typable in the SA tests. Three of these were from the new serogroups G, H and I respectively [6]. Two typable isolates in groups A to F failed to agglutinate, as did one isolate which has not yet been

successfully typed in AGDP. In future it should be possible to prepare specific agglutinating antisera against the three new serogroups by applying the same strategy of cross-absorbing the typing sera. It is not clear why the other isolates did not agglutinate, but it may reflect differences in expression of LPS components by different isolates.

Previous work by Lemcke and Bew [13] examined the possibility of using SA with absorbed sera to type *T. hyodysenteriae*, but they concluded that this was not practical because of the diversity of antigenic types of the bacteria that they encountered. This conclusion was arrived at because at that time *T. hyodysenteriae* was thought to occur only as a limited number of 'serotypes'. Examination of the results of Lemcke and Bew indicates that by using SA they were able to differentiate between strains S75/1, B169, P18A and KF9 (now recognized as being of serogroups B, C, D and E respectively). Furthermore by cross-absorbing sera against isolates having the same 'serotype' (serogroup), they were able to demonstrate antigenic differences between these isolates. In this case it appears that they were successfully using SA to identify individual serovars within the serogroups (e.g. B234 and B78 in serogroup A, B204 and S75/1 in serogroup B, and KF9 and MC52/80 in serogroup E).

The results of the current study, supported by the work of Lemcke and Bew [13], demonstrate that SA can be used as a rapid and simple means of typing isolates of *T. hyodysenteriae*. The availability of this technique should facilitate future epidemiological studies on swine dysentery.

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