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The serological grouping system for *Serpulina (Treponema) hyodysenteriae*

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SUMMARY

Lipopolysaccharide from serostrains of *Serpulina* (*Treponema*) *hyodysenteriae* for serogroups A to I was characterized using sodium dodecylsulphate polyacrylamide gel electrophoresis and silver staining. All strains had lipopolysaccharide components ranging from 10 to 16 kDa that represented lipid A-core polysaccharide regions, and short O-antigen side chain were also recognized in certain immunoblots.

Serological reactions between lipopolysaccharide and antisera against each of these serostrains were examined by Western immunoblotting. There was relatively little antigenic cross-reactivity between LPS from the nine strains, thus confirming their suitability as serostrains.

Using cross-absorbed sera, isolates within serogroups A and E were shown to possess unique epitopes on the core lipopolysaccharide, distinct from serogroup reactivities. These isolates were therefore identified as serovars within the serogroups.

This study confirmed the usefulness of the serotyping scheme for *S. hyodysenteriae*, in which the bacteria can be placed into serogroups using unabsorbed sera, and into serovars within these using cross-absorbed sera.

INTRODUCTION

Serpulina hyodysenteriae, previously known as *Treponema hyodysenteriae*, is an oxygen-tolerant anaerobic spirochaete [1–3], and is the primary aetiological agent of a major colonic disease of pigs, called swine dysentery [4, 5]. The outer envelope of spirochaetes, including *S. hyodysenteriae*, resembles that of Gram-negative bacteria, with lipopolysaccharide (LPS) forming a major component [6]. LPS extracted from *S. hyodysenteriae* is antigenic, and has been used to define serotypes of the bacteria. Initially, *S. hyodysenteriae* was classified into four serotypes based on serological reactions in agarose gel double immunodiffusion tests (AGDP) between LPS extracts and hyperimmune rabbit sera raised against whole formalinized spirochaetal cells [7]. Three other serotypes were subsequently described by Lemcke and Bew [8], whilst Mapother and Joens [9] published details of three more. The latter were defined using sera that were selectively cross-

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absorbed to remove activity against one or more of the original serotypes 1 and 2.

More recently the presence of more than one major LPs antigen in other isolates of *S. hyodysenteriae*, and the existence of complex cross-reactions between serotypes was reported [10]. To rationalize this situation it was therefore proposed that isolates of *S. hyodysenteriae* be organized into serogroups, each of which was represented by a type strain or 'serostrain'; unabsorbed antiserum raised against the serostrain could then be used to identify other isolates belonging to the serogroup [10]. Isolates within each serogroup that could be shown to possess additional epitopes using cross-absorbed sera were then called serovars. This system is analogous to that used for another spirochaete, *Leptospira interrogans*. A total of nine serogroups of *S. hyodysenteriae* (A to I) have been described to date [10–12].

Recently details of two new serotypes of *S. hyodysenteriae*, types '8 and 9', were published without any reference to their serogroup or to the existence of the serogrouping system [13]. The purpose of the present work was to demonstrate the validity and usefulness of the serogrouping system for *S. hyodysenteriae*, and thereby encourage its adoption.

MATERIALS AND METHODS

Microorganisms

The strains of *S. hyodysenteriae* used in this study are listed in Table 1, and have been described in previous publications [10–12].

Media and preparation of antisera

Growth of microorganisms in Trypticase Soy broth and production and absorption of antisera were as previously described [10].

LPS extraction

LPS was extracted by the hot water-phenol method, as adapted for *S. hyodysenteriae* [7]. However, the acetone precipitation step was omitted and instead the ethanol precipitated material was ultracentrifuged at 105000 g for 3 h at 4 °C. The clear gelatinous pellet containing purified LPS was then collected and resuspended in 0·5 ml of distilled water.

SDS–PAGE

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS–PAGE) of LPS from *S. hyodysenteriae* was conducted as described [14], except that a larger gel was used (16 cm × 20 cm × 1·5 cm). Electrophoresis was at 200 V (constant voltage) at 4 °C until the dye-front reached the bottom of the gel.

Silver staining

Selected gels were stained with silver using the protocol recommended by Bio Rad, slightly modified by the addition of 0·7 % periodic acid in the second fixative to produce a more sensitive detection of carbohydrates [15].

Coomassie blue staining

Selected gels were first fixed in 12 % trichloroacetic acid overnight. The gel was then stained with 0·05 % Coomassie brilliant blue R250 in a 10 % methanol, 7 %

Table 1. *Strains of* Serpulina hyodysenteriae *used in the study*

Strain	Serogroup	Origin
B78*	A	USA
WA15	A	Western Australia
WA1*	B	Western Australia
B169*	C	Canada
A1*	D	UK
WA6*	E	Western Australia
KF9	E	UK
MC52/80	E	UK
Vic1*	F	Victoria
Q16*	G	Queensland
Vic2*	H	Victoria
NSW1*	I	New South Wales

* Serostrains for serogroups A to I respectively.

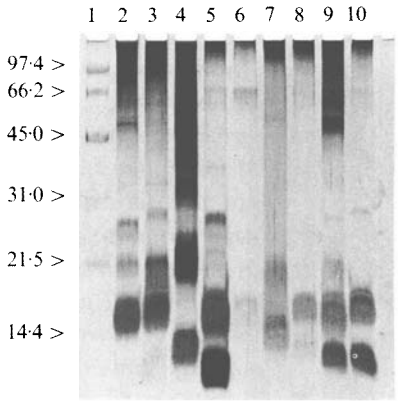

Fig. 1. Silver stained LPS profiles of serostrains of *S. hyodysenteriae*. Lanes from left to right: (1) molecular weight markers (kDa); (2) B78 (group A); (3) WA1 (group B); (4) B169 (group C); (5) A1 (group D); (6) WA6 (group E); (7) Vic1 (group F); (8) Q16 (group G); (9) Vic2 (group H); (10) NSW1 (group I).

acetic acid solution. Subsequently, gels were destained with 3·5% acetic acid until distinct bands appeared.

Western immunoblotting

Immunoblotting was as previously described [14], except that the transfer was carried out at 30 V (constant voltage) overnight at 4 °C using a Protean II xi transblot electrophoretic transfer cell (Bio Rad).

LPS from each of the nine serostrains for serogroups A to I were sequentially reacted with antisera raised against each of these strains. Isolates B78 and WA15 in serogroup A and MC52/80 and KF9 in serogroup E were each also reacted with unabsorbed sera prepared against both members of each pair, and with sera that was cross-absorbed with the other member of the pair [10].

RESULTS

The silver-stained LPS profiles of the nine serostrains of *S. hyodysenteriae* for serogroups A to I are shown in Fig. 1. Molecular weights were compared to protein molecular weight markers and were therefore only approximate. In these analyses,

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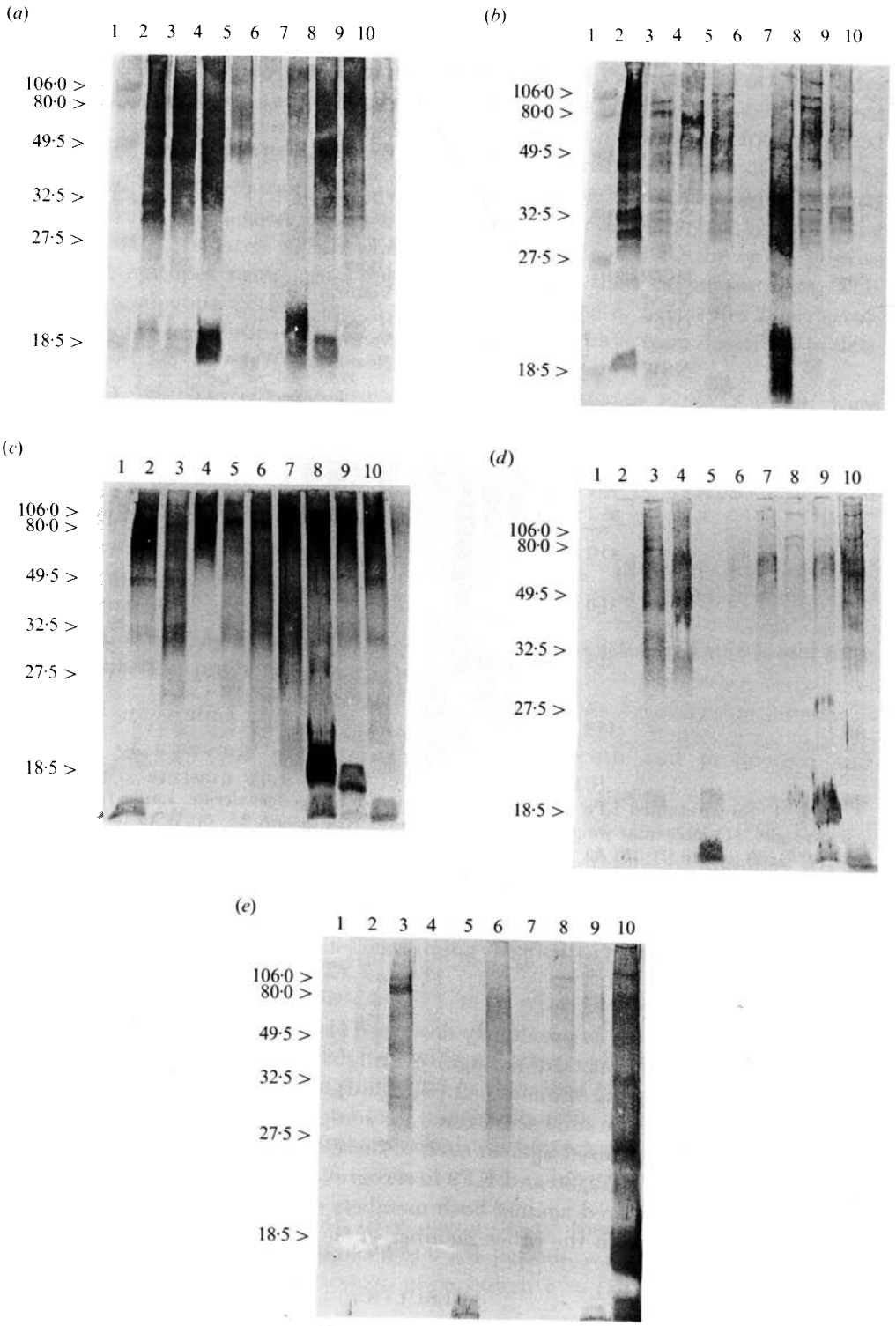

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any bands that had a molecular weight of 30 kDa or more were considered to be in the high molecular weight region, other components were in the low molecular weight region.

The major LPS components were of low molecular weight (Fig. 1), and stained orange/brown. Most of the serostrains had only one major broad LPS component. For B78, WA1, WA6 and Q16 this was at around 16 kDa, for B169 at 12 kDa and for Vic 1 at 14 kDa. A1 had major bands at approximately 16 and 10 kDa, whilst for Vic 2 and NSW1 these were at 16 and 11 kDa. B78, WA1, B169 and A1 in particular also had one or more additional bands between 20 to 30 kDa. All strains had a few faint bands with molecular weights about 50 kDa. None of these bands stained with Coomassie brilliant blue R250.

Immunoblot analyses of the serostrains for serogroups C, F, G, H and I of *S. hyodysenteriae* are shown in Fig. 2*a–e*. Results comparing serogroups A, B, D and E have previously been published [14], and there was little additional cross-reactivity with the other serogroups. In each case, each antiserum prepared against a serostrain of *S. hyodysenteriae* reacted strongly with low molecular weight material from the homologous strain. Some weak one-way (non-reciprocal) cross-reactions were, however, observed in the low as well as high molecular weight regions between certain strains. Serum against B78 (serogroup A) reacted feebly with low molecular weight components in Q16 (serogroup G). Serum against WA1 (serogroup B) reacted with similar material in Vic1 (serogroup F). Serum against B169 (serogroup C) cross-reacted with low molecular weight LPS from Q16 and Vic2 (Fig. 2*a*). Serum against A1 (serogroup D) reacted weakly with low molecular weight components in WA1 and WA6 (serogroups B and E respectively). Antiserum against WA6 (serogroup E) was specific, reacting only with its own low molecular weight LPS components (membrane not shown). Serum prepared against isolate Vic1 (serogroup F) reacted strongly with its own low molecular weight LPS components, weakly with the low molecular weight component of B78, and more clearly with high molecular weight material in all the preparations (Fig. 2*b*). Antiserum raised against Q16 (serogroup G) reacted with its major component at 16 kDa and weakly with a 10 kDa band, but cross-reactions also occurred with a highly mobile LPS band from B78 and with low molecular weight bands from strains Vic2 and NSW1 (Fig. 2*c*). Serum raised against Vic2 (serogroup H) reacted with homologous LPS bands at 16 and 11 kDa and cross-reacted with bands at around 10 kDa in isolates A1 and NSW1 respectively (Fig. 2*d*). Serum raised against NSW1 (serogroup I) reacted strongly with its own LPS components in low and high molecular weight regions, and cross-reacted weakly with low molecular weight bands in A1 and Vic2 (Fig. 2*e*).

Absorbed antisera were used to detect additional LPS epitopes on isolates within serogroups A and E. For serogroup A, unabsorbed antiserum raised against WA15 was shown to recognize and react with its own LPS, and with the 16 kDa component in the serostrain B78 (Fig. 3*a*); serum raised against isolate B78 also reacted with a similar component in WA15 (not shown). The absorption of serum

Fig. 2. Immunoblots of *S. hyodysenteriae* LPS (kDa). For each membrane, lanes from left to right: (1) molecular weight markers; (2) B78 (group A); (3) WA1 (group B); (4) B169 (group C); (5) A1 (group D); (6) WA6 (group E); (7) Vic1 (group F); (8) Q16 (group G); (9) Vic2 (group H); (10) NSW1 (group I). Membranes (a), (b), (c), (d) and (e) reacted with antisera against B169, Vic1, Q16, Vic2 and NSW1 respectively.

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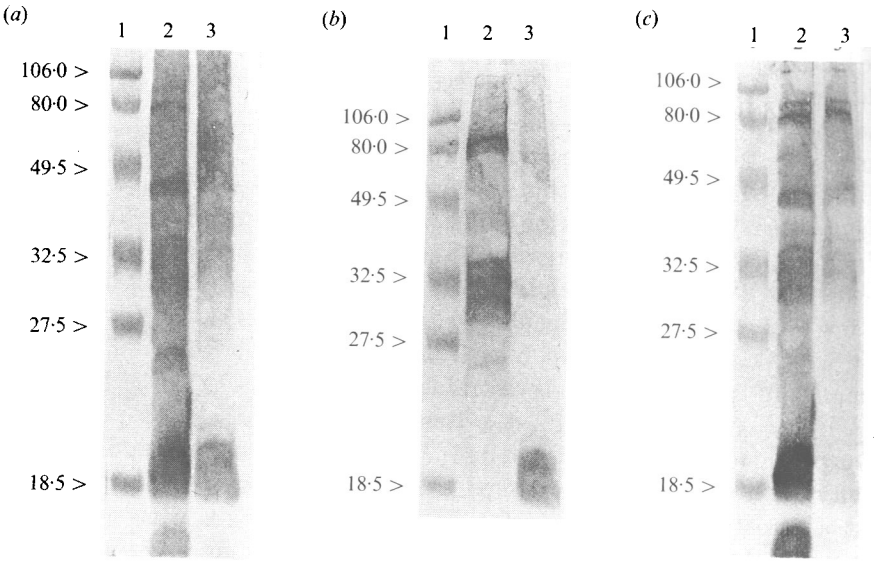

Fig. 3. Immunoblots of LPS from strains of *S. hyodysenteriae* of serogroup A (kDa). For each membrane, lanes left to right: (1) molecular weight markers; (2) B78; (3) WA15. Membranes (a), (b) and (c) reacted with antiserum against WA15, antiserum against WA15 absorbed with B78 cells, and antiserum against B78 absorbed with WA15 cells, respectively.

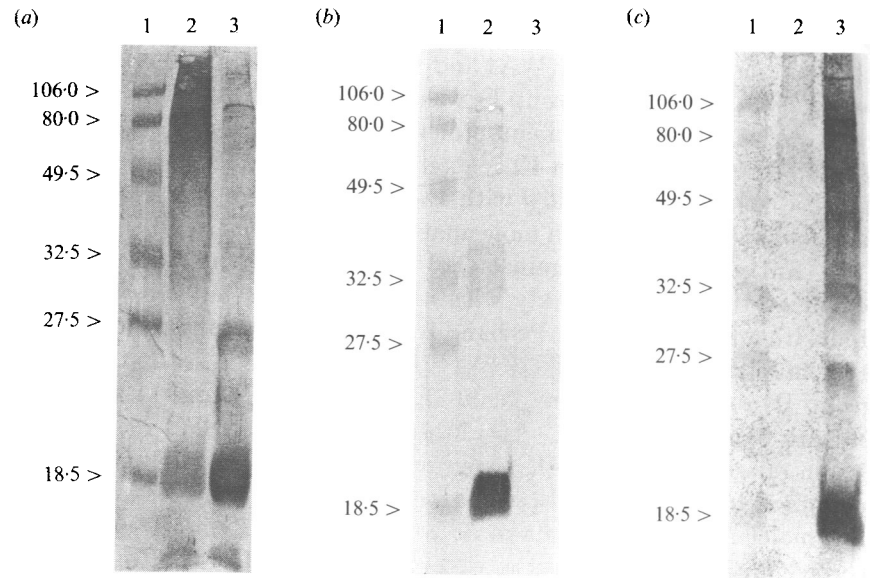

Fig. 4. Immunoblots of LPS from Strains of *S. hyodysenteriae* of serogroup E (kDa). For each membrane, lanes from left to right: (1) molecular weight markers; (2) KF9; (3) MC52/80. Membranes (a), (b) and (c) reacted with antiserum against KF9, antiserum against KF9 absorbed with MC52/80 cells, and antiserum against MC52/80 absorbed with KF9 cells, respectively.

raised against WA15 with B78 cells eliminated all activity against the low molecular weight LPS components from B78, but the serum still reacted with LPS from the homologous organism (Fig. 3*b*). When antiserum against B78 was absorbed with WA15 cells, it still reacted with all LPS components from B78, but

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activity against the low molecular weight LPS component from isolate WA15 was removed (Fig. 3*c*).

The existence of serologically distinct strains (serovars) were also demonstrated in serogroup E. Unabsorbed antisera raised against either KF9 or MC52/80 reacted with LPS bands in both the high and low molecular weight regions common to both isolates (Fig. 4*a*). However, when antiserum prepared against KF9 was absorbed with MC52/80 cells, it still reacted with KF9 but only showed weak reactions with high molecular weight LPS components of MC52/80 (Fig. 4*b*). Similarly, when serum prepared against MC52/80 was absorbed with KF9 cells it reacted with LPS from the homologous organism, but did not recognize LPS components from KF9 (Fig. 4*c*).

DISCUSSION

The silver stain profiles of LPS from the serostrains for the nine serogroups of *S. hyodysenteriae* all contained similar components in the low molecular weight region (Fig. 1). Similar profiles have been observed with other strains of *S. hyodysenteriae* [16–18]. By analogy with the Enterobacteriaceae, these low molecular weight LPS components (10–16 kDa) that stained orange/brown with silver appear to be the lipid A-core oligosaccharide region [19, 20]. The profiles of the faint bands in the high molecular weight region, particularly in certain immunoblot preparations (eg. Fig. 2*b*), showed a resemblance to the ladder-like LPS patterns which are generated by heterogeneity in the O-polysaccharide side chain in smooth LPS, and which are characteristic of LPS of the Enterobacteriaceae [21]. In these bacteria, the number of repeating oligosaccharide units can be as few as two (semi-rough type LPS) or as many as 40 (smooth-type LPS) [22]. Since there were relatively few repeating bands observed in the profiles of LPS from *S. hyodysenteriae*, these spirochaetes appear to have a semi-rough LPS. It was not clear why these components stained poorly with silver.

Typing sera prepared against each serostrain reacted most strongly with lipid A-core oligosaccharide components in the approximate range between 10 and 16 kDa from the homologous organisms. This is therefore the material that is responsible for serogroup specificity. There was minor non-reciprocal cross-reactivity involving a number of serostrains, but this reactivity is not detectable in AGDP [10–12]. These results indicate that the nine strains are appropriate serostrains for serogroups A to I.

Unabsorbed antiserum prepared against serostrain B78 was previously used to place WA15 into serogroup A [10]. In the current study, serum against B78 that was absorbed with WA15 cells, and serum against WA15 that was absorbed with B78 cells, were used to demonstrate that B78 and WA15 both possessed unique epitopes on their core LPS. These two isolates could, therefore, be regarded as being distinct serovars in serogroup A.

Lemcke and Bew reported that MC52/80 and KF9 were serologically different [8], and, using AGDP, Hampson and colleagues [10] identified these two strains as being distinct serovars in serogroup E. The current work, using Western immunoblotting, confirmed these results and also demonstrated that the unique epitopes belonging to these two stains reside on the core LPS.

The present study validates the serological typing scheme proposed by

Table 2. *Serological grouping of selected strains of* S. hyodysenteriae

	Serovars			
Serogroup	1	2	3	4
A	B78*	WA15	B6933	
B	WA1*	B204	B8044	ACK 300/8
C	B169*			
D	A1*	Q1		
E	WA6*	MC 52/80	KF9	
F	Vic1*			
G	Q16*			
H	Vic2*			
I	NSW1*			

* Serostrains.

Hampson and colleagues [10]. It demonstrates the specificity of the nine serostrains for serogroups A to I and the presence of serovars in serogroups A and E. Serovars in serogroup B have previously been demonstrated [10]. Reference strains B8044, B6933 and ACK 300/8 for serotypes 5, 6 and 7 as described by Mapother and Joens [9], were not available for testing, but could apparently be fitted into this serogrouping scheme. B8044 reacts with serum against B204 (a serovar in serogroup B [10]), but also has unique epitopes which can be detected with absorbed serum. It could, therefore, be regarded as a serovar in serogroup B. B6933 reacts with serum against B234 (a strain of serogroup A [10]), but also has unique epitopes. It therefore appears to be a distinct serovar in serogroup A. ACK 300/8 apparently reacts with sera against both B234 and B204, but also has unique epitopes [9]. It would therefore appear to span serogroups A and B. Lemcke and Bew [8], however, reported that this strain, under the name JWPM 300/8, belonged to 'serotype' 2 (equivalent to serogroup B). For this reason we favour placing ACK 300/8 into serogroup B, as a distinct serovar, but noting its potential cross-reactivity with strains from serogroup A. A summary of the current serogroups and serovars, amalgamated from previous results [10–12], the present work and the above considerations, is presented in Table 2.

Unfortunately, the relationships of 'new' serotypes 8 and 9 [13], represented by isolates FM 88-90 and FMV 89-3323 respectively, has not been established, other than to show that they do not belong to serogroups A to D. We suggest that, in the future when isolates of *S. hyodysenteriae* with 'distinct' serological properties are identified an attempt be made to integrate them into the serogrouping scheme outlined in Table 2.

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