The Serological Grouping of Three Strains of *Streptococcus equinus*

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SUMMARY

Group D antisera were prepared from three culture-collection strains of *Streptococcus equinus*. Reciprocal absorption tests showed that they were Group D streptococci.

INTRODUCTION

It is now well established that the streptococci are one of the predominant groups of organisms in the intestine of the pig (Raibaud & Caulet, 1957; Fuller *et al.* 1960). Consequently the classification of streptococci from this source has recently attracted much attention and many different species have been described as predominating in the pig gut. The type of organism isolated from mixed populations is always in some measure dependent on the medium used. Since at least 30 different media have been used by various workers (see Raibaud *et al.* 1961) it is not surprising that conflicting claims have been made. However, apart from the variation which can be accounted for in this way, there is the problem of classifying organisms which bear resemblance to more than one species. The lactose non-fermenting streptococci isolated from the pig gut are an example of this latter group of organisms. In previous work (Fuller *et al.* 1960) numerous lactose non-fermenting Group D streptococci were isolated from pig faeces; because of their inability to ferment lactose or to hydrolyse starch they were classified as *Streptococcus equinus*, although other workers had classified similar strains as *S. bovis* (Raibaud & Caulet, 1957). An objection to the classification of these lactose non-fermenting isolates as *S. equinus* was that they were of serological Group D, whereas no group specific antigen had so far been demonstrated for this species (Bergey's *Manual*, 1957). It was thus essential to know whether accepted strains of *S. equinus* were of serological Group D.

METHODS

Organisms examined. *Streptococcus faecalis* NCTC 775, *S. faecalis* var. *liquefaciens* (Elv. 2025) NCDO 588, *S. faecalis* var. *zymogenes* NCDO 586, *S. faecium* NCTC 7171, *S. durans* (98D) NCDO, 596, *S. bovis* (Pearl 11) NCDO 597, *S. equinus* ATCC 9812, NCDO 1090 and 1091. The three strains of *Streptococcus equinus* all conformed to the description given in Bergey's *Manual* (1957).

Media. For the preparation of vaccines and extracts organisms were grown for 24 hr. at 37° in glucose Lemco broth (% w/v; Evan's peptone, 1; Lab. Lemco, 1; NaCl (Analar), 0.5; glucose, 1).

Serological methods

Preparation of antisera. Antisera were prepared by using the three strains of *Streptococcus equinus* and the strain of *S. bovis*. Vaccines were prepared by the method of Shattock (1949). Saline suspensions of organisms were shaken in a tissue disintegrator (Mickle, 1948) for 90 min. One ml. was injected into the ear vein of rabbits at 3- to 4-day intervals, fresh vaccine preparations being used for each injection. Eleven doses of *S. equinus* and nine of *S. bovis* were given. Test bleedings were made after six injections and antisera were tested by the ring precipitin test against HCl extracts of streptococci of serological groups A-O.

Antigen extracts. These were prepared by the method of Lancefield (1933) and when necessary were concentrated by precipitation with ethanol as described by Shattock (1949).

Absorption of antisera. The method of Shattock (1949) was used.

RESULTS

In preliminary tests after six injections, the three *Streptococcus equinus* antisera gave no precipitation with any of the extracts of groups A to O streptococci whilst the *S. bovis* antiserum reacted weakly with the group D extract. At the end of the series of injections all four antisera reacted strongly with the extract prepared from *S. faecalis* 775, but when the sera were tested against extracts of the six different group D streptococci shown in Table 1 precipitation did not occur in all cases.

Table 1. *Precipitin reactions of Streptococcus equinus and S. bovis group antisera with normal and concentrated extracts of group D streptococci*

Antisera	HCl-extracts of											
	<i>S. faecium</i>		<i>S. faecalis</i>		<i>S. faecalis</i> var. <i>liquefaciens</i>		<i>S. faecalis</i> var. <i>zymogenes</i>		<i>S. durans</i>		<i>S. bovis</i> 597	
	N	C	N	C	N	C	N	C	N	C	N	C
<i>S. equinus</i>												
1090	—	+	+	+	+	+	—	+	—	+	—	+
1091	—	+	+	+	+	+	+	+	—	+	—	+
9812	—	+	+	+	—	+	—	+	—	+	—	+
<i>S. bovis</i>												
597	+	+	+	+	+	+	+	+	+	+	+	+

N, HCl extracts; C, concentrated HCl extracts.

However, when the extracts were concentrated the four antisera reacted with all the group D extracts but were still negative to extracts of the other serological groups. Therefore, in subsequent tests concentrated extracts were used.

To confirm that *Streptococcus equinus* was group D, reciprocal absorption tests were made (Tables 2, 3). When the group D antiserum prepared from *S. bovis* was absorbed with suspensions of *S. equinus* two absorptions were necessary to remove all the group D antibodies. After this treatment the only precipitin reaction which occurred was the type reaction between the *S. bovis* antiserum and the homo-

logous extract. One absorption was sufficient to remove the group D antibodies from the *S. equinus* antisera by using *S. faecalis* 775. The only remaining reactions were those between homologous systems. There was no evidence from either of these tests of any shared type antigens, no cross precipitation occurring between the three strains of *S. equinus* or between these and *S. bovis*

Table 2. *Absorption of group D antiserum by Streptococcus equinus suspensions*

Antigen; concentrated extract from	Group D serum (<i>S. bovis</i> 597)				
	Unabsorbed	Absorbed with			
		<i>S. equinus</i> 9812	<i>S. equinus</i> 1090	<i>S. equinus</i> 1091	<i>S. bovis</i> 597
<i>S. faecium</i>	+	—	—	—	—
<i>S. faecalis</i>	+	—	—	—	—
var. <i>liquefaciens</i>	+	—	—	—	—
var. <i>zymogenes</i>	+	—	—	—	—
<i>S. durans</i>	+	—	—	—	—
<i>S. bovis</i> 597	+	+	+	+	—
<i>S. equinus</i> 9812	+	—	—	—	—
<i>S. equinus</i> 1090	+	—	—	—	—
<i>S. equinus</i> 1091	+	—	—	—	—

Table 3. *Absorption of Streptococcus equinus and S. bovis group D antisera with S. faecalis 775*

Antigen; concentrated extract from	Antisera							
	<i>S. equinus</i> 9812		<i>S. equinus</i> 1090		<i>S. equinus</i> 1091		<i>S. bovis</i> 597	
	Unabs.	Abs.	Unabs.	Abs.	Unabs.	Abs.	Unabs.	Abs.
<i>S. faecium</i>	+	—	+	—	+	—	+	—
<i>S. faecalis</i>	+	—	+	—	+	—	+	—
var. <i>liquefaciens</i>	+	—	+	—	+	—	+	—
var. <i>zymogenes</i>	+	—	+	—	+	—	+	—
<i>S. durans</i>	+	—	+	—	+	—	+	—
<i>S. bovis</i> 597	+	—	+	—	+	—	+	+
<i>S. equinus</i> 9812	+	+	+	—	+	—	+	—
<i>S. equinus</i> 1090	+	—	+	+	+	—	+	—
<i>S. equinus</i> 1091	+	—	+	—	+	+	+	—

DISCUSSION

By using three culture collection strains of *Streptococcus equinus* we have prepared specific group D antisera thereby showing that *S. equinus* belongs to serological group D. It seems that the group D antigen is either serologically inaccessible or present in relatively small amounts as shown by the necessity to concentrate some extracts to get precipitation with *Streptococcus equinus* group antisera and the necessity to absorb twice with *S. equinus* suspensions to remove group D antibodies from a *S. bovis* group antiserum. This agrees with our previous findings (Fuller *et al.* 1960) that *S. equinus* strains isolated from pig faeces were difficult to group unless concentrated extracts were used. It may also explain past failures to detect a group specific antigen.

Since this work was completed Smith & Shattock (1962) have reported on a

collection of streptococci isolated from horse faeces and corresponding to the description of *Streptococcus equinus* (Andrewes & Horder, 1906). These strains all produced the group D antigen and the preparation of a group D antiserum from one of these strains confirmed their opinion that *S. equinus* belongs to group D.

Group D is at present comprised of *Streptococcus faecalis* and its two variants *liquefaciens* and *zymogenes*, *S. durans*, *S. faecium* and *S. bovis*. These organisms are all of intestinal origin and since *S. equinus* is the predominant streptococcus in horse faeces (Andrewes & Horder, 1906) it is perhaps not surprising that, if it belongs to any of the existing groups, it should belong to group D. The similarity of the physiological characters of *S. equinus* and *S. bovis* (Sherman, 1937) supports the inclusion of *S. equinus* in group D. Seeley & Dain (1960) stated that aside from the inability of *S. equinus* to ferment lactose, no significant differences exist in our present definitions and knowledge of the species. This close relationship to *S. bovis* coupled with a reluctance to place group D organisms in a species for which no group antigen was known may have led to the classification of *S. equinus* isolates as *S. bovis*. The acceptance of *S. equinus* as a member of serological group D removes the latter difficulty. More recently Smith & Shattock (1962) have re-examined the relationship between the two species and suggest that when the physiological characters of each species are viewed as a whole, then a satisfactory separation can be made. We also believe that these two species can be separated on the basis of physiological tests and would propose that the species *S. equinus* be retained and in future included in group D.

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