STREPTOCOCCUS EQUINUS

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Received for publication October 12, 1936

Andrewes and Horder (1906) discovered as the predominating streptococcus occurring in air a type which did not ferment lactose. As horse dung made up a large part of the organic pollution of the London air of that time, they suspected this material as the source of the organism. An investigation of fresh horse dung confirmed their suspicion, this particular organism being not only the principal streptococcus found but usually making up a majority of the total bacterial flora.

This organism, which they named *Streptococcus equinus*, was described by Andrewes and Horder as being devoid of pathogenic properties, non-hemolytic, and having the following additional characteristics: milk is not coagulated; there is little or no reducing action on neutral red; sucrose, salicin and coniferin are usually fermented; lactose and mannitol are not fermented; raffinose and inulin are not attacked as a rule, but a number of variant types which ferment these substances were found. Andrewes and Horder also pointed out the important fact that *S. equinus* has a high minimum temperature of growth, evidenced by little or no growth in gelatin cultures at 20°C. They considered horse dung the chief source of the organism but thought that it might occur in the intestines of other herbivora. They did not succeed in obtaining it from the intestines of certain carnivora examined—the fox and the stoat.

An excellent early contribution to the subject was the work of Winslow and Palmer (1910) which verified the findings of Andrewes and Horder and reported the finding of *Streptococcus equinus* in the intestines of the cow and of man. Those occurring

in the human intestine were believed to represent a distinct variety, marked by its high fermentative power in glucose broth. Among other relatively early contributors to this subject were Fuller and Armstrong (1913) and Broadhurst (1915).

So far as we are aware, there has been no significant contribution to the knowledge of this organism since the early investigations, and with the passing of the years the name *Streptococcus equinus* has become a convenient "wastebasket" into which have been thrown streptococci that do not ferment lactose. Its "identification" has been simplified in the systems of classification. For example, Blake (1917) classified non-hemolytic streptococci which fail to ferment lactose and mannitol as *S. equinus*; while Holman (1916) used the same three tests with the addition of salicin, those failing to ferment salicin being named *Streptococcus ignavus*.

Streptococci failing to ferment lactose have been reported from many sources, including the mouth, throat, urine and feces of humans. Arnold (1920), for instance, found a large proportion of the streptococci isolated from human throats to be unable to ferment lactose. Also, Floyd and Wolbach (1914) and others have isolated non-hemolytic streptococci from human infections, which did not ferment lactose. It is desirable to know whether these widely distributed organisms are in fact *S. equinus*, or if additional species are involved, or, on the other hand, if many of the organisms which fail to ferment lactose are simply aberrant strains of other species rather than the true *S. equinus* of Andrewes and Horder.

The present investigation was undertaken in order to obtain a more adequate description of *Streptococcus equinus* through the use of the more extensive and modern methods now available for the study of the streptococci. A total of 72 cultures which did not ferment lactose, isolated from 20 samples of fresh horse dung, were studied in detail. As in the experiences of former workers, this type was found to be the predominating organism in the intestinal material from the horse.

The various tests employed are mostly well known procedures in the study of streptococci and all of them have been described

in previous papers. For the fermentation tests, the test materials were sterilized separately in 10 per cent solutions and later added to the sterile broth. Mannitol and glycerol were sterilized by heat, the other test substances by filtration.

The reported characteristics were determined on all of the 72 cultures with the exception of their actions on esculin and fructose. After the investigation had been completed and only 15 of the cultures were available, it seemed desirable to test the remaining cultures on esculin because of the supposed importance of this substance in connection with *Streptococcus fecalis* and its relatives among the "enterococci." At the same time, we tested the remaining cultures for action on fructose. The hexose sugars have not been shown to have differential value in the study of streptococci, but, in this case, galactose was included as having possible interest because the organisms involved do not ferment lactose. The tests conducted with the remaining cultures on fructose showed, as was to be expected, that this sugar is also fermented.

Esculin was found to be acted upon, but slowly. When tested after three days' incubation at 37°C. a number of the cultures gave negative reactions, but these negative cultures all gave positive reactions after ten days. Upon retests made in triplicate, it was again found that some cultures were negative after two days, a few still negative after four days, but all were positive after seven days' incubation.

THE CHARACTERISTICS OF STREPTOCOCCUS EQUINUS

Morphology. The characteristic grouping is in short chains, the chains usually being somewhat longer in broth cultures than in milk. Some cultures form extremely long chains in broth.

Blood. In horse-blood agar the alpha type of reaction is given. The degree varies somewhat with different cultures, some giving a weak, but nevertheless definite, reaction.

Minimum temperature of growth. No growth occurs at 10°C. nor at 15°C. At 21°C. very slow growth takes place, requiring about three weeks to give a slight acidity in glucose-peptone-litmus milk, a medium in which the organism grows with especial vigor.

Maximum temperature of growth. Growth takes place at 45°C. but there is no growth at 48°C. Growth seldom occurs at 47°C. (three of 72 cultures).

Thermal resistance. Only ten of the 72 cultures proved able to withstand a heat treatment of 60°C. for 30 minutes in milk. This suggests a somewhat higher resistance than is possessed by the pathogenic streptococci (Ayers, Johnson and Davis, 1918), but substantially lower than that of the thermoduric streptococci (Sherman and Stark, 1931).

Litmus milk. No visible change is produced in litmus milk.

Litmus milk + 2 per cent glucose. With added glucose, litmus milk is rendered acid but is rarely curdled (four of 72 cultures) and there is little reduction of the litmus.

Final pH. In glucose broth, final pH values of 4.4 to 4.1 are attained.

Sodium hippurate. Sodium hippurate is not hydrolyzed.

Starch. Starch is not hydrolyzed under the conditions of the test used. (This test was considered of interest because in some respects *Streptococcus equinus* appears to be related to *Streptococcus bovis* which hydrolyzes starch actively. In three attempts, using both ordinary nutrient agar and yeast-peptone as bases, no growth was obtained on the starch agar plates.)

Ammonia. Ammonia is not produced in 4 per cent peptone.

Esculin. Esculin is hydrolyzed slowly.

Gelatin. Gelatin is not liquefied. (These tests were conducted at 37°C.)

Fermentation reactions. Glucose, fructose, galactose and maltose are fermented.

Sucrose (66 or 72 cultures) and salicin (63 of 72 cultures) are usually fermented.

Raffinose (four of 72 cultures) and inulin (22 of 72 cultures) are usually not fermented.

Arabinose, xylose, lactose, mannitol and glycerol are not fermented.

Viability. *Streptococcus equinus* is readily lost from artificial cultures with infrequent transfers, ranking in this respect with freshly isolated cultures of *Streptococcus thermophilus* and *Strepto-*

coccus salivarius. In this connection, however, it should be recalled that Andrewes and Horder showed *S. equinus* to be very resistant to drying.

DISCUSSION

It is believed that the somewhat extended description of *Streptococcus equinus* here presented shows it to constitute a fairly clearly defined species quite aside from its failure to ferment lactose. Its very high minimum temperature of growth and its high maximum temperature of growth, combined with a relatively low thermal resistance; its feeble action in 2 per cent glucose litmus milk, together with little reducing action; its inability to hydrolyze sodium hippurate or starch, produce ammonia from peptone, or ferment arabinose, xylose, glycerol or mannitol; these, with its general pattern of reactions with other tests, mark fairly clearly the natural boundaries of the species. The variations noted in the fermentation of sucrose, raffinose, inulin and salicin correspond exactly with the findings of Andrewes and Horder. We agree with Andrewes and Horder that there is as yet no sound basis for the division of this group into additional species.

It is probably unnecessary to labor the point, but since divisions of this group on the basis of certain fermentation tests have been suggested, a few further remarks may be pertinent. There were nine cultures which were atypical on salicin, six on sucrose, four on raffinose, and 22 on inulin.. In the case of inulin the variant cultures constituted nearly one-third of the collection, but among these strains there were none which were atypical on sucrose or raffinose and three which were atypical on salicin, almost precisely what would be expected from random distribution. Since salicin has been suggested as a differential test, it may be noted that among the variants on this substance none were atypical on sucrose, two on raffinose, and three on inulin. Of the atypical cultures on sucrose none were variant in other respects, except one that fermented raffinose. The only possible correlation which could be considered abnormal from the standpoint of chance distribution, was in the case of two cultures

which were among the four raffinose fermenters, and also among the nine which did not ferment salicin. It would be humorous to suggest these two cultures as constituting a type, but, even if this were done, it would leave remaining 50 per cent of raffinose-fermenting cultures, and 77 per cent of those that failed to ferment salicin, which were not otherwise atypical.

As was previously noted, Holman classified the salicin-negative strains as a new species, *Streptococcus ignavus*. Since Andrewes and Horder defined *S. equinus* as sometimes failing to ferment salicin, such a departure would hardly appear justified in the absence of other correlating characteristics. Bergey (1934) adopts Andrewes and Horder's *S. equinus* and Holman's *S. ignavus* but violates the authors' descriptions in both cases. Aside from the salicin reaction, he describes *S. equinus* as not fermenting raffinose or inulin, while the ability to ferment these substances is ascribed to *S. ignavus*. Andrewes and Horder showed that *S. equinus* sometimes ferments raffinose or inulin, while Holman made no use of raffinose and only one of his 71 cultures of *S. ignavus* fermented inulin. It should be recalled that Andrewes and Horder reported a total of 95 atypical cultures of *S. equinus*, isolated from various sources, which fermented either raffinose or inulin; and that Holman states that *S. ignavus* was so named because of its lack of action on the test substances used.

We do not mean to imply that there is only one species among the non-hemolytic streptococci which do not ferment lactose; only that additional species have not yet been clearly defined. In fact, it would appear from the literature that a detailed study of those organisms obtained from such sources as the human throat and human infections might prove well worth while.

Related to this general problem is the question of how frequently aberrant strains, which do not ferment lactose, occur among the ordinary streptococci. Such are well known to occur even in a strong lactose-fermenting type like *Bacterium coli*, and numerous cultures of *Streptococcus pyogenes*, or cultures that have been so classified, have been reported which lacked this ability. In this laboratory, we have recently had atypical strains of *Streptococcus lactis* and *Streptococcus salivarius* which did not

ferment lactose, but which could be clearly differentiated from *S. equinus*.

Another question is whether or not atypical forms of *S. equinus* which do ferment lactose are of frequent occurrence. We have made no special study to determine this point. It may be pertinent to record, however, that in making this collection 20 lactose-fermenting cultures of streptococci were obtained from fresh horse feces. Eighteen of these were definitely identified as *S. fecalis*, but the other two cultures, though not *S. fecalis*, were not sufficiently studied to allow conclusions concerning their species identity.

It is hoped that the present work may prove of some value in giving a more extensive description of *Streptococcus equinus* and in defining slightly better the boundaries of the species.

SUMMARY

A detailed study was made of 72 cultures of *Streptococcus equinus* isolated from fresh horse feces. The cultures were studied by the application of most of the newer methods which have been found of value in the differentiation of streptococci, in addition to the fermentation tests. It is believed that the data obtained will serve to indicate fairly clearly the natural boundaries of the species.

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