Relationships of Group E streptococci to swine throat abscesses

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RELATIONSHIPS OF GROUP E STREPTOCOCCI
TO SWINE THROAT ABScesses

by

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A Dissertation Submitted to the
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The Requirements for the Degree of
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1955
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In the United States there is a disease or some characteristic in

Intrusion
includes the soft tissues of the ventral and lateral neck region of the pig from the angle of the mandible to the thoracic inlet.

Regardless of the name applied to the disease it is one of growing concern to farmers, veterinarians, and meat packers. The presence of throat abscesses in young swine intended for sale as breeding stock forces the farmer to sell them for slaughter. Owners of herds where the disease is endemic may find their sales of market hogs restricted to a "subject to inspection" basis. Veterinarians are concerned with control measures of the disease, however, no effective control measures have been reported. Meat packers are concerned because this disease condition is the leading cause of swine carcass parts condemned due to an infectious disease (24). At slaughter, under federal inspection, the condemned heads (and necks) are removed from the carcasses and sent to the rendering tank.

A survey by Pickard (19), including a number of meat packing plants located in various regions of the United States, showed condemnations of abscessed heads at slaughter to be as high as 7 per cent of the total hog kill in some plants. An instance was cited in which the abcessed heads of 80 carcasses from a consignment of 111 hogs were condemned on postmortem inspection. The loss due to condemnations was estimated at $120.00 ($1.50 per head condemned). The operators of one plant of moderate slaughtering capacity in Eastern Iowa (17) estimated that they suffered an annual loss of at least $50,000 due to condemnations of swine heads because of throat abscesses.

In view of the foregoing, it is evident that swine throat abscesses are of considerable significance to all parties concerned. Surely a
disease of such importance is worthy of investigation with a view toward its control and eventual eradication.

The published reports of other workers regarding the disease and regarding the Group E streptococcus are briefly reviewed below. The research conducted by this writer is presented in two sections. The first chapter deals with the cultural survey of nearly 500 swine throat abscesses obtained from various sources. The second chapter includes a series of experiments using pigs as test animals and Lancefield's Group E streptococcus as the test organism.
REVIEW OF LITERATURE

The few published reports concerning swine throat abscesses and the bacteria associated with such lesions are, with one exception, confined to reports originating in the United States during the past 20 years. The information contained in these reports is largely limited to clinical observations, incidence of the disease in infected herds, descriptions of gross lesions, and bacteriological findings.

Under the title, "Strangles in Hogs," Hutyra and Marek (12) described a condition observed by Starcovici in Roumania in 1898. It was a disease of young hogs characterized by inflammatory swelling followed by suppuration of the submaxillary and subparotid lymph nodes. The disease lasted several weeks, fever and emaciation were noted, but nearly all pigs recovered. On microscopic examination of smears of the exudate bipolar bacilli and streptococci were observed. Treatment consisted of draining the abscesses and flushing the cavities.

Newsom (16), working in Colorado, described an outbreak of throat abscesses that occurred during September in a group of 30 March-farrowed pigs. The affected animals coughed, wheezed, and showed multiple ventral cervical swellings. Multiple throat abscesses were encountered at autopsy of two animals that had shown neither depression of activity or elevated rectal temperatures clinically. The lesions ranged from the size of a walnut to that of a baseball and contained odorless, yellowish-green exudate under pressure. Pure cultures of a beta hemolytic
streptococcus were obtained from the exudate. The organism was not grouped serologically and may or may not have been a strain of the Group E streptococcus on the basis of the biochemical findings reported.

Stafseth and Clinton (21) first reported the isolation of a Lancefield Group E beta hemolytic streptococcus from swine cervical abscesses. The specimen examined was the head of a seven-month old sow obtained at slaughter. The animal came from a herd troubled for "some time" with throat abscesses. No clinical observations were reported. Six abscesses approximately the size of walnuts were found in the submaxillary and subparotid regions. They contained thick, creamy, greenish exudate. Each of three abscesses yielded a pure growth of a beta hemolytic streptococcus. That organism was studied with regard to cell morphology and staining, cultural characteristics, biochemical properties, and serologic grouping. It was pointed out that heretofore the isolation of Group E streptococci had been reported in a very limited number of instances from certified milk. Contact with dairy cattle and the feeding of unpasteurized milk to the swine were eliminated as possible sources of the infection. It was suggested that carelessness in connection with hog cholera vaccination may have been responsible for inducing the abscesses.

Collier (5) isolated a strain of the Group E streptococcus from abscessed cervical lymph nodes of each of three swine at autopsy. The identification of the organism was based on its cultural characteristics, biochemical reactions and serologic grouping.

Snoeyenbos (20) recovered a strain of the Group E streptococcus from each of five diseased swine from one herd. The animals were fed raw
The author doubted that carefulness vaccination of
by previous workers, the authors doubted that the results obtained
of the streptococcus were made and the results compared with those obtained
were no evidence of growth on other microorganisms, pure culture studies
satisfactorily stated by various methods revealed a few cocci; there
were no proof of such transport. Direct smears of extract from
where the chromatogenic and streptococcus were suggested between species.
met the chromatogenic and streptococcus. It was concluded that,
to that time were negative for the streptococcus. The experiment that,
to the development of the disease on those taken sometimes subsequent
not taken at that time; however, quarter samples taken sometime previous
were tested from the disease extract. Quarter samples for culturing were
membranous abscesses. These chromatogenic and streptococcus and streptococcus
a better pasteurized agent to the hot lot developed multiforme large,

though, weird, yellow-green, non-porous pus.

encephalitis abscesses measured 7 to 9 cm. In diameter and containing
encephalitis abscesses measured 7 to 9 cm. In diameter and containing
and asymptomatic in all other respects. The lesions consisted of blisters
were noted shortly thereafter. Apparently, this effect appeared normal
ventral cervical region on handling at vaccination. Well-developed abscesses

into the infected brains at 6x weeks or age showed swelling in two
in two successive years, one lot of pigs were 9 or 10 weeks of age (introduced
from that point was estimated at 40 per cent and 50 per cent, respectively;
period of three years, the incidence of the disease in vaccinated animals
in successive lots of the encephalitis in the Garbage feeding operation over a
the other encephalitis were removed. There was a history of cervical abscesses
purpoase pigs at six weeks or age and add them to the herd before all of
Garbage supplemented by some pasteurized skim milk. The practice was to
the affected pigs was a factor in the spread of infection in that outbreak.

Collier (6) reported an incidence of 94.7 per cent of jowl abscesses at slaughter in a herd of swine in northern Iowa. This outbreak occurred on a well-managed institutional farm where the sanitation was good. No garbage was fed, no milk or milk products were fed, and there were no known contact(s) between swine and cattle. A search of the premises accessible to the swine revealed no sharp objects that might cause trauma of the throat tissues of those animals. Abscesses were observed in pigs in that herd as early as 6-8 weeks of age and in adult breeding stock; however, such lesions were more common in shoats during the interval from weaning age to market weight. The nature of the gross lesions was essentially identical to that reported by earlier workers. Superficially located lesions were observed to point and then drain spontaneously through an opening in the overlying necrotic skin. Following drainage, a lesion slowly healed leaving a lump of dense fibrotic tissue in its place.

Beta hemolytic streptococci of Lancefield's Group E were isolated regularly from exudate taken from the abscesses of swine in the affected herd. Escherichia coli, Pasteurella multocida, and Corynebacterium pyogenes were occasionally obtained on culture in addition to the streptococcus. Acid-fast-stained smears of exudate were consistently negative for tubercle bacilli. Depopulation of the infected herd of hogs, disinfection of the premises, and the introduction of supposedly abscess-free breeding stock failed to eradicate the disease from the swine on that farm.
Lancefield (13) established the serologic Group B of streptococci using three cultures isolated by Brown (5) from certified milk. Coffey (4) reported the identification of 11 cultures of Group B beta hemolytic streptococci. Milk from cows in four different herds was the source of all but one of the cultures. One strain was isolated together with staphylococci from a wound on the hand of a dairy worker.

Hare (11) reported the isolation of a Group B beta hemolytic streptococcus from the mammary exudate of a sow the sixth day postpartum. The exudate was a yellowish-green, glutinous pus. The sow farrowed live pigs but suffered acute mastitis; the pigs were all dead by the third day after farrowing. If the infection of the mammary gland of the sow with the Group B streptococcus were common in herds where the throat abscess disease is enzootic, it would seem likely that the incidence of the disease would be higher than it appears to be in pigs less than two months of age.

Swift (22) reported that streptococci of serologic Group B were occasionally obtained as "carriage strains" or "pathogenicity undetermined" from cattle, dogs, and swine. Collier (5) isolated a culture of Group B streptococcus that was associated with bacteremia in a hog and a second culture associated with pneumonia in another animal of the same species. Glässer (9) in discussing swine diseases due to streptococci in Germany does not refer to throat abscesses.

Wellman (25) expressed the opinion that the throat abscess disease described in the United States does not exist in Germany. Boutet (2) is representative of a number of French workers that refer to systemic infections of young pigs due to streptococci, but do not mention that
genus of microorganism in connection with throat abscesses. Grina (10) states that Group E streptococci are not associated with pyogenic processes of domestic animals.
CULTURAL SURVEY OF SWINE THROAT ABScesses

Methods of Procedure

The 492 specimens obtained for culturing consisted of 438 unopened abscesses from meat packing plants and 54 specimens of exudate from abscesses of live swine in the field. All specimens of packing plant origin were divided into two lots. Lot No. 1 included 310 abscesses from one plant in eastern Iowa. Lot No. 2 included a total of 128 abscesses from three consignments of about equal size originating in southern Minnesota, northern Iowa, and central Ohio. The specimens from packing plants were lesions trimmed from the throat region of diseased swine by federally-employed inspectors at the time of slaughter. Each abscess was wrapped in grease-proof paper and then placed in an inner container with numbers of its kind. Refrigerant cans were included in the outer container along with insulating material in a generally successful effort to maintain the contents of the inner container in a fresh chilled condition during shipment.

Fifty-four specimens of exudate from field cases were included in lot No. 3. Those specimens were obtained by aseptically aspirating the exudate into a hypodermic syringe using a No. 16 gauge (or larger) hypodermic needle or by thrusting a sterile swab into the exudate at the time of surgical drainage.

Lesions of swine raised in Illinois and Iowa.
Direct smears were prepared in duplicate from 10 per cent of all specimens. In each instance one smear was stained by the acid-fast method and one by Gram’s method. All specimens were initially cultured on 5 per cent bovine blood agar. Exudate that was not typical with regard to color and odor was also streaked on sodium azide-crystal violet agar medium developed by Packer (18) as a selective medium for streptococci. The concentrations of sodium azide and crystal violet used in the medium were 1/2000 and 1/500,000, respectively. Cultures selected at random were incubated anaerobically at 37°C. Ten per cent of all specimens were also cultured on Sabouraud’s medium. Those cultures were incubated at room temperature. All other inoculated media were incubated at 37°C. Colonial growth characteristics were observed after 24, 48, and 72 hours of incubation. Smears of representative colonies were stained by Gram’s method prior to microscopic examination. Other standard methods were used to obtain growth of the selected organisms in pure culture and in the inoculation and interpretation of results in the differential media used for the various kinds of bacteria encountered.

The battery of carbohydrate media for the identification of the Group B streptococcus included lactose, mannite, raffinose, salicin, sorbitol, trehalose, and imulin broths plus litmus milk. Five per cent (random selection) of all cultures that were identified as Lancefield’s Group B streptococcus on the basis of cultural characteristics, cellular morphology, hemolysis, and biochemical reactions were also subjected to serological grouping. Part of those cultures were grouped by Dr. Updyke (23) and part by this writer. In the latter instance the procedure
employed was a combination of the micro precipitin method developed by Lancefield (14) and the formamide extraction method developed by Fuller (8).

Appropriate differential media, as indicated by Bergey (1) and/or Merchant (15), were used in the identification of other kinds of bacteria. Strains of Pasteurella multocida were tested for pathogenicity for mice by inoculating 0.1 ml. of broth culture of that organism intraperitoneally into mice.

The objective in culturing the various specimens was to isolate, identify, and tabulate the kinds of bacteria that occurred with a view toward selecting any that might be of significance insofar as the etiology of swine throat abscesses is concerned. The criteria for the selection of significant organisms derived from cultures of abscess exudate were, 1) high overall incidence, 2) regular occurrence among various lots of specimens, and 3) reputation as a pyogenic species of microorganism in swine.

Results

The abscesses obtained from the packing plants ranged from less than 0.5 cm. to more than 10 cm. in diameter. The capsules consisted of dense, white fibrous connective tissue and varied in thickness from less than 1 mm. in tiny abscesses to more than 10 mm. in the large lesions. Many of the lesions had evidently been trimmed from superficial locations beneath the skin. The overlying skin was frequently necrotic.

The exudate was characteristically light green in color, non-odorous,
and fluid to semi-solid in consistency. It contained leucocytes, necrotic tissue debris, and bacteria. Occasional specimens of exudate also contained granules of calcareous material. Now and then a specimen was pinkish, brownish, or yellowish instead of the usual greenish color. Some of these specimens had a very foul odor. Invariably these atypical exudates yielded bacteria other than the Group E streptococcus, however, occasional exudates of the characteristic greenish, odorless type yielded mixed growth on blood agar.

Two small abscesses (2.0 to 3.0 cm. diameter) contained a microorganism that was identified as *Actinomyces bovis* on the basis of its morphology in stained smears. The lesions yielding that organism on smears were not typical of those yielding the Group E streptococcus. On cross section the capsule surrounded a spongy mass of material which appeared to be lymphoid tissue undergoing liquefaction necrosis. The usual greenish color of the exudate was missing from those lesions. Material from those abscesses inoculated into deep agar medium enriched with dextrose and serum failed to produce growth, and there was no growth on aerobically-incubated media.

No evidence of *Nocardia tuberculosis* was obtained on examination of the acid-fast preparations. No fungi grew on the Sabouraud's medium. Media incubated anaerobically yielded no kinds of growth in addition to those obtained on aerobically-incubated duplicate media.

On blood agar the Group E streptococcus grew slowly and showed little evidence of hemolysis after 24 hours of incubation, however, at 48 hours the isolated colonies measured 0.8 mm. in diameter and
were surrounded by sharply defined circular zones of beta type hemolysis measuring about 1.5 to 2.0 mm. in diameter. The colonies were circular with entire margins and a glossy surface. In profile they were globular (i.e. high, single convex). The interiors of the colonies were milky opaque, however, the surface layer or covering was translucent. Occasional cultures on blood agar were overgrown by proteus or crowded with various kinds of bacterial growth. The sodium azide crystal violet medium was invaluable in isolating the streptococcus in such instances. In a few instances where Pasteurella multocida and the Group E streptococcus shared the same exudate, growth of the latter organism was completely suppressed on blood agar but grew on the azide medium. The same strains of Past. multocida, however, when subcultured on a blood agar plate with the streptococcus showed no inhibitory effect.

The cultures of the Group E streptococcus grew readily in beef infusion broth medium forming chains of 3 to 16 cells. There was some variability in diameter of occasional cells in some chains. No capsules were detected in preparations of cells from either liquid or solid media. The growth in broth was characterized by uniform turbidity, no pellicle formation, and slight to moderate precipitation of cells. Acid production in certain of the carbohydrate media was usually apparent after 24 hours of incubation. The group E streptococcus characteristically produced acid, but no gas, in mannite, salicin, sorbitol, and trehalose broths; but not in lactose, raffinose, or imulin broths (Table 1). Occasional strains were slow or negative in producing acid from sorbitol. Almost without exception the streptococcus produced a transitory reduction in litmus milk that was apparent after 16 to 24 hours of incubation but not at 48 hours. No
other change was noted in that medium.

It is apparent on inspection of Table 1 that other workers did not obtain reduction in litmus milk or that the reaction may have been overlooked because of its transitory nature. There is complete agreement on the reaction obtained in lactose for cultures of swine origin (Bergey's characteristics are based on reactions of strains isolated from milk).

Table 1. Comparison of physiological characteristics of Group E streptococci as reported by various workers

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Collier</th>
<th>Snoeyenbos</th>
<th>Stafseth</th>
<th>Newsom</th>
<th>Bergey's manual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus milk</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>acidified</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>coagulated</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>reduced</td>
<td>+\textsuperscript{b}</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mannite</td>
<td>+</td>
<td>+</td>
<td>+\textsuperscript{c}</td>
<td>+</td>
<td>+\textsuperscript{a}</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salicin</td>
<td>+</td>
<td>-</td>
<td>+\textsuperscript{a}</td>
<td>-</td>
<td>+\textsuperscript{a}</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>+\textsuperscript{d}</td>
<td>+\textsuperscript{c}</td>
<td>+\textsuperscript{a}</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Imulin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Acid production variable.

\textsuperscript{b} Transient.

\textsuperscript{c} Reaction appearing only after 48 or 96 hours.

\textsuperscript{d} Occasional strains slow or negative in acid production.
There is good agreement on reactions in mannite, raffinose, sorbitol, trehalose, and inulin broths, however, there is disagreement on reactions in salicin broth. In the hands of this writer the Group E streptococcus consistently produced acid in salicin.

There was 100 per cent agreement between the biochemical and serologic identifications obtained by this writer in all instances where the colonial growth was typical of that previously described for the Group E streptococcus.

The most significant result of the cultural survey was the consistent isolation of Lancefield's Group E streptococcus from all classes of specimens. There was a total of 421 isolations of that organism from the 492 specimens cultured. The breakdown in numbers of isolations of that streptococcus from the various lots is shown in Table 2. The higher percentage of both pure cultures and total isolations of that organism

Table 2. Incidence of pure cultures and total isolations of Group E streptococcus from the exudate of swine throat abscesses

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Sources of abscesses cultured</th>
<th>Number of abscesses cultured</th>
<th>Pure cultures of Group E streptococcus</th>
<th>Total isolations of Group E streptococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>packing plant A</td>
<td>310</td>
<td>217 (70%)</td>
<td>281 (90.6%)</td>
</tr>
<tr>
<td>2</td>
<td>plants B, C, and D</td>
<td>128^</td>
<td>65 (50.8%)</td>
<td>88 (68.8%)</td>
</tr>
<tr>
<td>3</td>
<td>various field cases</td>
<td>54</td>
<td>42 (90.7%)</td>
<td>52 (96.3%)</td>
</tr>
<tr>
<td>Total:</td>
<td></td>
<td>492</td>
<td>331 (67.3%)</td>
<td>421 (85.6%)</td>
</tr>
</tbody>
</table>

^Seven abscesses in this group yielded no bacterial growth.
from the lesions in lot No. 1 as compared to lot No. 2 was associated
with a more careful selection of the specimens in the former lot to avoid
excessive numbers of lesions in the later stages of development (i.e. those
with necrotic overlying skin). The highest incidence of both pure cultures
and total isolations of the streptococcus and the lowest incidence of
other kinds of bacteria was obtained in lot No. 3.

The following list includes the generic and specific name and the
overall incidence of each of 11 kinds of bacteria other than the Group E
streptococcus that were isolated from the exudate of swine throat
abscesses: Corynebacterium pyogenes (12.8%), Pasteurella multocida
(5.89%), Proteus ammoniae (3.2%), Streptococcus equisimilis (1.42%),
Escherichia coli (1.02%), Streptococcus zooepidemicus (0.81%), Actinomyces
bovis (0.41%), Proteus vulgaris (0.20%), Salmonella typhimurium (0.20%),
and Staphylococcus aureus (0.20%).

Corynebacterium pyogenes was isolated from each of the lots of
specimens. It occurred occasionally in pure culture, but more frequently
it was obtained from a mixed culture in combination with one or more of
the microorganisms listed above or with the Group E streptococcus. In
lot No. 1 the corynebacterium was frequently associated with exudate from
lesions in the advanced stage of development or with those adjacent to an
abscess that had drained.

Pasteurella multocida was also isolated from each of the lots of
specimens and occurred both in pure culture and in mixed cultures. It
was obtained from clinical cases of the throat abscess disease that were
characterized by a marked, diffuse swelling of the throat region during
the initial stages of the disease. Five cultures of Paste. multocida, three cultures of Coryn. pyogenes, a single culture of Escherichia coli, and 52 cultures of the Group E streptococcus constituted the entire bacterial flora obtained from the 54 specimens in lot No. 3.

Streptococcus equisimilis and Streptococcus zoosporadicus were obtained in pure or mixed cultures, however, neither of them were isolated from mixed cultures that also contained the Group E streptococcus. There were occasional specimens of exudate that yielded kinds of bacteria that were not completely identified. Streptococcus spp., Bacillus spp., Corynebacterium spp., Alkaligenes spp., and Micrococcus spp. were included in that category. These organisms were most frequently associated with abscesses having necrotic overlying skin.

Discussion of Results

The agreement between the findings of this writer and those of other workers regarding the nature of the abscesses suggests that all are considering the same disease and that it is widespread in the United States. Similar general agreement regarding the various characteristics of the bacterium designated as Lancefield's Group E streptococcus suggests that all are referring to the same microorganism. The consistent isolation of the group E streptococcus, by this writer and others, from the exudate of swine throat abscesses originating in widely separated regions of the country, suggests that it is usually present in such lesions regardless of the geographic source of the diseased animal(s).

In order to qualify as a possible etiologic agent of an infectious
disease a microorganism must be regularly associated with that particular condition. The Group E streptococcus with an overall incidence of 85.6 per cent is the only microorganism, in the work reported above, that came close to meeting that requirement. Furthermore, it would seem from the cultural results obtained in lot No. 3 that the incidence of the Group E streptococcus isolated from throat abscess exudate is a function of selection of the specimens to be cultured. The total number of isolations of the streptococcus indicated that there is a large reservoir of that organism in swine in the United States.

Corynebacterium pyogenes is a recognized pathogen that is associated with suppurative inflammation in swine, however, it must be excluded as a significant primary etiologic agent of throat abscesses because of its low incidence (12.8%) in these lesions.

Pasteurella multocida, Streptococcus equisimilis, and Streptococcus zooepidemicus can certainly be excluded from consideration as significant primary etiologic agents of the swine throat abscess disease on the basis of the low overall incidence of each of those species in the survey, however, all of them are recognized pathogens and are associated with suppurative processes in swine. It would seem likely that occasional throat abscesses may be induced by these microorganisms.

While Actinomyces bovis and Spherophorus necrophorus are pathogens that may be associated with suppurative lesions in swine, both may be excluded as significant etiologic agents of throat abscesses because of their exceedingly low incidence in those lesions and because of the atypical exudate associated with their presence.
In the following chapter, experiments of that sort were performed and are reported.

normal saline. Experiments of a pure culture of the organism to be induced by the administration of a pure culture of that organism.

In microbial experiments to determine whether or not the disease could be induced by the administration of a pure culture of that organism.

The microbial experiments to determine the pathogenic agent of that organism was group B streptococcus in the primary etiological agent of that organism.

The strong induction, gained as a result of the culturial survey,

with suppressive process in syringe

end or lack of suppression of pathogens, and/or lack of known association

since the pathogenesis disease on the basis of interference or coincidence

may be excluded from consideration as possible etiologic agent of the

All remaining kinds of microorganisms observed in the culturial survey

-20-
INFECTIVITY EXPERIMENTS USING THE GROUP E STREPTOCOCCUS AS THE TEST ORGANISM* AND THE PIG** AS THE TEST ANIMAL

Experimental Procedures

The Group E streptococcus inoculum for administration to pigs was, with one exception, prepared from strain CD52. That strain was used exclusively in the limited trials involving rabbits and mice. Strain CD52 was isolated in pure culture from exudate taken from a throat abscess of a market hog at slaughter. That animal was farrowed and raised to market weight on a northern Iowa farm where the disease was endzootic.

A total of 49 pigs were used in the various experiments. They were weaned animals of both sexes, of various breeds, and had initial weights of 40 to 60 pounds. Animals known to be disease-free were obtained from the Veterinary Research Institute at Iowa State College for use in all instances except in the bacterin prophylaxis experiment. In that instance pigs were purchased from another source, and they proved unsatisfactory because they were not disease-free. The pigs were confined in concrete-floored pens indoors. Good pen sanitation was maintained, however, in one instance sanitation was neglected purposely; the waste feed, urine,

*Corynebacterium pyogenes and Pasteurella multocida were also used as test organisms in one experiment.

**Mice, rabbits and a guinea pig were also used, in a very limited way, as test animals.
or two titles in each of which the group of streptococcus pneumoniae red
experiments were denoted by letter. Experiments on certain enzymes were
for the purposes of organization and reporting of the work, the various
were listed in appropriate places below.

excesses), aged heart's blood, procedures used only in certain experiments
were studied repeatedly and the cut surfaces observed for evidence of
excesses in the tissues of that region. All lymph nodes of that
and excessive, special attention was directed toward the detection of
logic internal to the various experimental animals were destroyed.

excesses were subjected from developing excesses for culturing.


do of the thoracic region was used to detect enlarged lymph nodes, and
detect atrophy in the physis of the test animals. Postpartum
to detect atrophy of the thyroid gland, the test animals were used
during the postpartum period to
in some instances, the number of counts were noted, and,
for culturing these species. The behavior of the phis of the phis was noted, and,

group is streptococcus. The sodium acetate-cysteine shorter medium was used
withneath. During this period the range of rectal temperature readings
in pens and allowed a period of some days for them to adjust to their new
contents at the start of an experiment were to place the animals to be used
recees, and dirty bedding were allowed to accumulate. The routine pro-
to pigs. Trial No. 1 was a preliminary exploration, while trial No. 2 confirmed the previous findings and yielded some additional results. In the latter trial the cultural examinations were extended to include blood, feces, and skin of the inoculated animals, the feed trough in which the inoculum was fed, the pen filth, and (at autopsy) stomach contents, visceral organs, and visceral lymph nodes.

Blood for culturing was drawn from the anterior vena cava. Five ml. of freshly-drawn citrated blood was added to 50 ml. of tryptose broth containing 1 per cent dextrose. Broths that showed evidence of growth after incubation were subcultured on blood agar. Fecal material, swabs from a feed trough, and swabs of pen filth were streaked on the sodium azide-crystal violet medium. Skin in the axillary region was cultured by passing a sterile, moistened swab over an area of it before and after light scarification and then using the swab to inoculate blood agar and sodium azide-crystal violet medium.

Experiment B (Table 4) was designed to obtain information on the degree of contagiousness of the Group E streptococcus. It consisted of two trials in which no inoculum was fed or given by any route. In trial No. 1 of that experiment two normal pigs were placed in a recently-vacated, uncleaned pen in which the Group E streptococcus inoculum had been fed to other pigs three weeks previously. The previous occupants of that pen had developed throat abscesses, however, no abscesses had drained in that pen. In the second trial, two normal animals were placed in a disinfected pen for a two week contact period with an animal showing visible throat abscesses. The latter animal was scrubbed with a quaternary
ammonium disinfectant solution before being placed in the contact pen. The throat lesions of that animal did not drain during the contact period.

The object of Experiment C (Table 5) was to discover what methods of administration of the inoculum, other than in feed, might be effective in inducing throat abscess formation. Two pigs were inoculated intragastrically and each of six other pigs was inoculated by a different route including intravenous, intrapharyngeal, intranasal, subcutaneous, intraperitoneal, and intraenteric. The intragastric and intraenteric inoculations were accomplished by injecting the inoculum through the walls of the organs with a hypodermic syringe and No. 25 gauge needle after they were exposed by making a surgical incision through the abdominal wall on the ventral midline. Intranasal and intrapharyngeal inoculations were accomplished by expelling inoculum from a hypodermic syringe onto the mucosa of those areas. The remaining sites were injected with the aid of a hypodermic syringe and a No. 20 gauge hypodermic needle. In addition to the routine postinoculation observations and tests, cultures were made of the arterial blood of the pig (WSF) inoculated intravenously. The blood was obtained from that animal by tail bleeding. In each of the remaining animals the cultural examination was extended to include the site of inoculation and the contiguous lymph nodes.

Experiment D (Table 6, Fig. 2) was performed to get information regarding the immunizing value of a Group E streptococcus bacterin against infection and throat abscess induction by the homologous strain of that organism. Three pigs were placed in each of three pens. The pigs in pen No. 1 were inoculated subcutaneously on two occasions with the bacterin,
those in pen No. 3 received a single injection of the bacterin intravenously, and the animals in pen No. 2 served as unvaccinated controls. All pigs were later challenged with live culture inoculum of the same strain (D41) of the Group E streptococcus used in preparation of the bacterin.

The bacterin used in that experiment consisted of a formalin-killed, 24-hour whole broth (tryptose with 1 per cent dextrose) culture of strain D41 of the Group E streptococcus. That strain was selected at random from among the pure cultures of the organism that were isolated from the specimens contained in lot No. 2 above. The cells in the bacterin were adjusted to a concentration of approximately 2 billion per ml. A rapid plate agglutination test was devised to detect increases in anti-group E streptococcal agglutinins in the blood sera of the pigs during the postvaccinal and postchallenge periods. The antigen used in the rapid plate test consisted of formalized Group E streptococcus (strain D41) cells suspended in normal saline in proportions of 0.02 ml. of packed bacterial cells per ml. of fluid.

Serum to be tested was pipetted onto a glass plate in amounts of 0.08 ml., 0.02 ml., 0.01 ml., 0.005 ml., and 0.001 ml. The latter two quantities were pipetted after the serum was diluted 10 fold. Antigen in a constant volume of 0.02 ml. was added to each site where serum was placed and to a control containing 0.02 ml. of normal saline only. The serum and antigen at each site was mixed, and the smears were examined for evidence of agglutination after 10 minutes of incubation at room temperature.

Experiment B (Table 7) was conducted to determine whether or not
The proposed criterion for determining the aqueous suspension. The proposed criterion for determining the aqueous suspension of (17000 units/ml) in a standardized preparation was one comprising benzylpenicillin (17000 units/ml). Three units per milliliter were given as a single injection of penicillin to three of 7 days, and three at 16 days. The penicillin concentration, three at 7 days, was considered evidence of infection.

Three units per milliliter were given as a single injection of penicillin to three of 96 to 90 hours. Penicillin concentration was considered evidence of infection of the mucous of the group B streptococcus. Preparing during the interdialysis of the mucous of the group B streptococcus. The bacterial counts were substantially reduced at the mid culture serum levels of the antibiotic. Greatly in excess of 0.046 units per hour on the 1st day. The total levels were less than 4 and showed the presence of the antibiotic.

Penicillin were determined in the plasma following the injection of the antibiotic on a 0.046 units per ml. No blood levels were determined of penicillin or penicillin concentration. Penicillin or penicillin concentration in the plasma of the group B streptococcus were determined in the experiment. The growth of the group B streptococcus was inhibited in the experiment under experimental conditions. It was determined at the outset of the experiment that the group B streptococcus therapy in mice infected with the group B streptococcus were performed to gain some indication of the antibiotic of the antibiotic results.

Two experiments were performed from the other experiments. The former of the above two plus the group B streptococcus (strain CD266). The former experiment was red first, followed with penicillin. A second pen of the penicillin was red 0.046 units per ml. According to the pen were red pen of the group B streptococcus would induce the formation of the plaque of the group B streptococcus. Red separated on in mixtures with the group B streptococcus. Red separated on in mixtures of other organisms. Penicillin of the group B streptococcus was red separated on in mixtures with the group B streptococcus.
effectiveness of the treatment was whether or not cervical lymphadenitis or throat abscesses developed in the treated animals. Routine procedures were used to determine the postinoculation results.

Adult mice were inoculated intraperitoneally with a 24 hour beef infusion broth culture of strain CD52 in amounts ranging from 0.1 to 0.5 ml. A rabbit was inoculated intravenously with 1.0 ml. of that inoculum. A second rabbit received a total of 8 ml. of that inoculum by that route at the rate of 2 ml. per day over a 4 day period. A third rabbit received a series of intradermic injections (in skin of the back). Each site in one linear series received 0.1 ml. of the strain CD52 inoculum, each site in a second series received a like amount of cell free filtrate of that inoculum, and each site in a third received a like amount of sterile, normal saline. A guinea pig was inoculated orally with 2 ml. of 24 hour broth culture of strain CD52, and 0.5 ml. of the same inoculum was injected subcutaneously in that animal.

Results

Throat abscesses developed in pigs that received the Group E streptococcus culture in feed (Table 3) or by intranasal or intrapharyngeal instillation, and the test organism was recovered in pure culture from the exudate of those lesions. Animals inoculated by other routes did not develop throat abscesses and, with one exception noted below, did not develop abscesses elsewhere in the body. The test organism was frequently isolated from pharyngeal mucus, tonsillar tissue, and occasionally from nasal mucus of animals in which throat abscesses
<table>
<thead>
<tr>
<th>Time</th>
<th>CD4 Positive</th>
<th>CD4 Negative</th>
<th>CD8 Positive</th>
<th>CD8 Negative</th>
<th>CD19 Positive</th>
<th>CD19 Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>60 days</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 3:** Results of Feeding Leneetfeld’s Group 1 Strain cultures to mice.
developed, however, it was impossible to recover it from any site in a	number of those inoculated by other routes.

The clinical indications of disease and the findings at necropsy were
quite similar in all cases in which throat abscesses developed, and certain
general observations can be made regarding them. During an interval of
at least 36 hours postinoculation there were no significant changes in the
appearance of the test animals. Leucocyte counts and rectal temperature
readings remained within preinoculation ranges during that period. There
was variation among pigs with regard to the onset of pyrexia, but some-
time during the interval from 36 to 96 hours postinoculation there was a
rapid, high elevation of body temperature (Fig. 1). There was a con-
current rapid rise in the leucocyte count due, largely, to a marked in-
crease in the numbers of immature neutrophiles. The decline of the
initial pyrexia, after 3 or 4 days was paralleled by a drop in the
leucocyte count. During the first day of the febrile period anorexia
and depression of activity of the inoculates were frequently observed,
however, animals showing those symptoms were back on feed a day later
and gave every outward indication of being normal pigs until about 3
weeks later when visible swellings of the throat region indicated that
abscesses were developing.

Elevated body temperature and leucocytosis occurred at irregular
intervals after the sixth day postinoculation, but they were of lesser
magnitude than the initial rises and were not associated with anorexia
and/or depression of activity of the diseased animals. While visible
evidence of abscess development was not apparent until about 21 days
FIG. 1. CORRELATION BETWEEN PYREXIA AND LEUCOCYTOSIS IN PIGS FED GROUP E STREPTOCOCCUS INOCULUM
postinoculation, careful palpation of the throat region revealed swollen mandibular lymph nodes as early as the fifteenth day postinoculation. Superficially-located abscesses were observed to induce pressure necrosis of the overlying skin during the fifth week postinoculation. Exudate from lesions covered with necrotic skin yielded mixed growth on cultures.

Since none of the experimental animals died as a result of disease, they were destroyed at various times for the purpose of postmortem examination. At autopsy the diseased animals had 1 or more abscesses within the soft tissues of the throat region. Those lesions ranged from less than 1.0 cm. to more than 7 cm. in diameter, were heavily encapsulated, and contained a greenish, non-odorous exudate which varied in consistency from that of thin cream to that of thick paste. The gross appearance of the abscesses induced under experimental conditions were indistinguishable from those seen on the farm or in the meat packing plant. The smaller abscesses (0.2 to 2.0 cm.) were invariably found embedded in the lymph nodes of the throat region. The mandibular lymph nodes were most commonly involved, however, the retropharyngeal and parotid lymph nodes were also common sites for abscess development. No lymph node tissue could be identified in association with the larger abscesses.

The principal findings in Experiment A are listed in Table 3. In trial No. 1 each of three pigs developed throat abscesses following the feeding of broth culture of the Group E streptococcus. Pigs W52 and B52 developed large bilateral lesions near the ventral midline of the throat between the angles of the mandibles. Pig R52 developed a large lesion in the left parotid region. Each of the 3 animals also had 1 or more
smaller lesions involving lymph nodes adjacent to the large lesions.

The pathogenesis of throat abscesses was partially defined as a result of certain findings in the second trial of Experiment A. Pig B53 showed a marked elevation of both rectal temperature and leucocyte count at 36 hours postinoculation (Fig. 1), however, on autopsy at 48 hours postinoculation no gross evidence of an inflammatory process could be found anywhere in the body, except that some of the cervical region lymph nodes showed a moderate degree of congestion. The test organism was recovered on cultures of pharyngeal swabs taken at 6 and 12 hours postinoculation, but it was not recovered from any source in that animal at autopsy.

Pig GB53 evidenced the initial febrile period following inoculation. At autopsy on the seventh day postinoculation the mandibular lymph nodes were hyperemic, swollen, and contained scattered miliary abscesses that could be seen beneath the node capsule and on the cut surface. The Group B streptococcus was recovered in mixed culture from tonsillar tissue and in pure culture from the tiny abscesses.

The onset of postinoculation pyrexia in pig W53 was evident at 48 hours and persisted at least 120 hours thereafter. Just prior to autopsy on the fifteenth day the swollen, turgid mandibular lymph nodes could be readily palpated. The actual surface dimensions were 4.0 cm. by 2.0 cm. by 1.5 cm. in each case. The cut surfaces revealed some thickening of the node capsule and multiple abscesses measuring 0.5 to 1.0 cm. in diameter. Interspersed among the abscesses was some grossly normal lymphoid tissue. The test organism was recovered from the pharynx of the live
animal, from abscess exudate, and from tonsillar tissue.

Pig BR53 developed a single large abscess on the ventral midline of the throat between the angles of the mandibles. The developing lesion was detected by palpation on the fifteenth day postinoculation, and pronounced necrosis and discoloration of the skin overlying the abscess was observed during the fifth week postinoculation. The remaining pig, RG53, in that group showed postinoculation pyrexia and leucocytosis, however, no evidence of throat abscesses was detected clinically or at autopsy.

The Group B streptococcus was not recovered from feces of infected animals, from pen filth, or from skin. It was recovered from the feed trough on the 3 consecutive days immediately following administration of the inoculum in the feed. It was not recovered from blood, except that taken during the 24 hours following the intravenous inoculation of pig WSP. Sites within the animal body other than the naso-pharynx, cervical region lymph nodes, and abscess exudate failed to yield that organism on culture.

None of the animals in the contact exposure trials of Experiment B (Table 4) gave any evidence, either clinically or at necropsy, of throat abscesses. The test organism was recovered from a grossly normal mandibular lymph node of one animal used in the pen contact trial.

Results of Experiment C are summarized in Table 5. Postinoculation pyrexia developed regardless of the route of inoculation or the volume of the inoculum, however, there was considerable variation in the length of the interval between inoculation and the onset of pyrexia depending on the route of inoculation. Cervical lymphadenitis and throat abscesses occurred only in animals inoculated either by the intranasal or intra-
Table 4. Contact exposure trials

<table>
<thead>
<tr>
<th>Pig identification</th>
<th>Trial No.</th>
<th>Method of exposure</th>
<th>Pyrexia</th>
<th>Leucocytosis</th>
<th>Anorexia and depression</th>
<th>Interval start of exposure to autopsy</th>
<th>Throat abscesses encountered at autopsy</th>
<th>Test organism from any site</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB53</td>
<td>1</td>
<td>pen contact with diseased pig</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>43 days</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BR53</td>
<td>1</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>43 days</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BRB53</td>
<td>2</td>
<td>dirty pen</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>51 days</td>
<td>-</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NB53</td>
<td>2</td>
<td>contact</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>57 days</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>Slight.

<sup>b</sup>Group E streptococcus recovered from grossly normal mandibular lymph node.
<table>
<thead>
<tr>
<th>Identification</th>
<th>Amount of inoculum and route of inoculation</th>
<th>Postinoculation pyrexia Onset</th>
<th>Postinoculation pyrexia Duration</th>
<th>Interval from inoculation to autopsy</th>
<th>Throat abscess(es) at autopsy</th>
<th>Abscess(es) elsewhere</th>
<th>Test organism recovered from any source</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSP</td>
<td>30 ml., intravenous</td>
<td>5 hrs.</td>
<td>8 days</td>
<td>16 days</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BL54</td>
<td>10 ml., subcutaneous</td>
<td>5 hrs.</td>
<td>96 hrs.</td>
<td>22 days</td>
<td>-</td>
<td>+</td>
<td>*a</td>
</tr>
<tr>
<td>RD54</td>
<td>10 ml., intraperitoneal</td>
<td>5 hrs.</td>
<td>96 hrs.</td>
<td>20 days</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HR54</td>
<td>5 ml., intrapharyngeal</td>
<td>48 hrs.</td>
<td>120 hrs.</td>
<td>28 days</td>
<td>+</td>
<td>-</td>
<td>*b, c</td>
</tr>
<tr>
<td>WH54</td>
<td>1 ml., intranasal</td>
<td>60 hrs.</td>
<td>108 hrs.</td>
<td>17 days</td>
<td>+</td>
<td>-</td>
<td>*b, c</td>
</tr>
<tr>
<td>RM54</td>
<td>10 ml., intraenteric</td>
<td>60 hrs.</td>
<td>48 hrs.</td>
<td>21 days</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WF54</td>
<td>10 ml., intragastric</td>
<td>60 hrs.</td>
<td>36 hrs.</td>
<td>21 days</td>
<td>-</td>
<td>-</td>
<td>*c</td>
</tr>
<tr>
<td>NT54</td>
<td>10 ml., intragastric</td>
<td>60 hrs.</td>
<td>48 hrs.</td>
<td>21 days</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*aAt site of inoculation in fold of flank.

*bAbscess exudate.

cTonsillar tissue.
pharyngeal routes. The degree of the systemic reactions and the size of
the lesions that developed in the pig (W54) inoculated intranasally
were similar to those that occurred in other animals receiving a much
larger volume of inoculum. The animal (SL54) inoculated subcutaneously
developed a single, heavily encapsulated abscess approximately 3 cm. in
diameter at the site of inoculation in the fold of the flank. The
exudate from that lesion was creamy in consistency, greenish in color,
and non-odorous. It yielded the test organism in pure culture. The
lymph nodes of the flank region were grossly normal and did not yield
the test organism on cultures.

Throat abscesses did not develop, and the test organism could not be
recovered at autopsy from any site in the body of pigs inoculated intra-
venously, intraperitoneally, intraenterically, or intragastrically,
except that the Group E streptococcus was isolated from tonsillar tissue
of one of the animals that was inoculated intragastrically.

The significant findings in Experiment D, concerning the use of
bacterin, are listed in Table 6. The pigs used in that experiment were
unthrifty. At autopsy there was marked atrophy of turbinate bones in all
of them. There is evidence, however, that they did respond to the
bacterin injections and to challenge with the live culture by developing
detectable increases in anti-Group E streptococcal agglutinins (Fig. 2).
None of the animals that were injected subcutaneously with bacterin
developed throat abscesses subsequent to challenge, however, two of the
untreated controls also failed to develop lesions. The three pigs
receiving bacterin intravenously developed slightly higher levels of
agglutinin following vaccination, but two of them developed postinocula-
Table 6. Attempted prophylaxis of the throat abscess disease in swine using Group E streptococcus bacterin

<table>
<thead>
<tr>
<th>Pen No.</th>
<th>Amount per pig</th>
<th>Route</th>
<th>Number of doses</th>
<th>Interval between doses</th>
<th>Volume of inoculum per pen</th>
<th>Pig identification</th>
<th>Post-challenge pyrexia</th>
<th>Throat abscess(es) at autopsy</th>
<th>Test organism recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 ml.</td>
<td>s.c.</td>
<td>2</td>
<td>10 days</td>
<td>100 ml.</td>
<td>MS&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;,c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MB&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WB&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
<td>100 ml.</td>
<td>SE&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;,c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LE&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BE&lt;sup&gt;4&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+&lt;sup&gt;b,c&lt;/sup&gt;d</td>
</tr>
<tr>
<td>3</td>
<td>5 ml.</td>
<td>i.v.</td>
<td>1</td>
<td></td>
<td>100 ml.</td>
<td>TB&lt;sup&gt;4&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BK&lt;sup&gt;4&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LT&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-</td>
<td>+&lt;sup&gt;f&lt;/sup&gt;</td>
<td>+&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Subcutaneous.
<sup>b</sup>From tonsillar tissue.
<sup>c</sup>From nasal mucus.
<sup>d</sup>From abscess exudate.
<sup>e</sup>Intravenous.
<sup>f</sup>Miliary in size.
FIG. 2. POSTVACCINAL AND POSTCHALLENGE TITERS OF ANTI-GROUP E STREPTOCOCCAL AGGLUTININS IN PIG BLOOD SERA.
tion pyrexia and sizable throat abscesses. The third animal showed no postinoculation pyrexia but did develop miliary abscesses. The test organism was recovered frequently from nasal mucus and tonsillar tissue of the animals in all 3 pens.

None of the animals fed broth culture of either Coryn. pyogenses or Past. multocida developed postinoculation pyrexia or any other symptoms of disease, and none showed throat abscesses at autopsy (Table 7). Postinoculation pyrexia and throat abscesses developed in each of 3 animals fed a mixture of culture of those organisms together with Group E

Table 7. Results of feeding Corynebacterium pyogenes or Pasteurella multocida culture to swine

<table>
<thead>
<tr>
<th>Pen number and pigs per pen</th>
<th>Volume per pen</th>
<th>Inoculum</th>
<th>Postinoculation pyrexia</th>
<th>Throat abscesses at autopsy</th>
<th>Test organism recovered from abscesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1 (4 pigs)</td>
<td>100 ml.</td>
<td>Coryn. pyogenes</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. 2 (3 pigs)</td>
<td>100 ml.</td>
<td>Past. multocida</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. 3 (5 pigs)</td>
<td>300 ml.</td>
<td>mixed&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>100 ml., ea., of Coryn. pyogenes, Past. multocida, and the Group E streptococcus.

<sup>b</sup>The Group E streptococcus was recovered in pure culture from exudate of the various throat abscesses encountered in each of the 3 animals at autopsy.
streptococcus culture. The clinical syndrome and the character of those lesions were no different than those seen in cases where the latter organism was fed in pure culture.

Pigs fed Group E streptococcus inoculum and subsequently treated with penicillin failed to develop throat abscesses (Table 8). Each of them showed postinoculation pyrexia before any penicillin was given. The duration of the initial postinoculation febrile period in animals receiving penicillin at 72 hours postinoculation was appreciably shortened as compared to the duration of that period in untreated pen mates (Fig. 3).

Table 8. Administration of penicillin to swine fed Group E streptococcus culture

<table>
<thead>
<tr>
<th>Group number and pigs per group</th>
<th>Post-inoculation interval before treatment</th>
<th>Amount of penicillin suspension per pig</th>
<th>Throat abscesses developed</th>
<th>Test organism recovered at autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1 (5 pigs)</td>
<td>72 hours</td>
<td>2 ml.</td>
<td>-</td>
<td>+b</td>
</tr>
<tr>
<td>No. 2 (5 pigs)</td>
<td>7 days</td>
<td>3 ml.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. 3 (3 pigs)</td>
<td>16 days</td>
<td>4 ml.</td>
<td>-</td>
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*Pigs are grouped according to treatment received, not as pen mates.

bFrom nasal mucus.
FIG. 3. COMPARISON OF THE POSTINOCULATION RECTAL TEMPERATURE CURVE OF A PIG GIVEN A SINGLE INJECTION OF PENICILLIN WITH THOSE OF UNTREATED PEN MATES.
None of the mice inoculated intraperitoneally with the Group E streptococcus culture died as a result of that inoculation. All of them remained sleek-coated, bright eyed, and active. No gross lesions were detected in any of the visceral organs of those mice at autopsy 10 days postinoculation, and the test organism was not recovered from peritoneal fluid, heart's blood, or kidney.

The rabbit that received 1 ml. of inoculum intravenously gave no observable indication of disease during a two week postinoculation period of observation. That animal was not sacrificed for autopsy. The rabbit that received a total of 8 ml. of the inoculum intravenously died the twelfth day postinoculation. Clinically it showed anorexia, dehydration, and progressive emaciation. At autopsy the visceral organs revealed lesions of a septicemic disease. Much kidney damage was apparent in the form of infarction and necrosis. The test organism was recovered from various sites in that animal.

The intradermic injections of whole culture inoculum in the case of a third rabbit induced areas of congestion and edema at the site of injection within 24 hours. Those areas reached a maximum diameter of about 2.5 cm. at 48 hours. At 7 days postinoculation fluctuant pustules measuring 0.5 to 1 cm. in diameter were apparent at the site of injection. At 14 days postinoculation these lesions were diminished in size and the remaining exudate was caseous. None of these lesions were observed to drain spontaneously. An attempt to recover the test organism from the exudate of these lesions at 7 days postinoculation was unsuccessful. No swelling was apparent at 24 hours postinoculation in areas
where either the cell-free filtrate or the saline was placed, and no changes were seen in those areas at any subsequent time within the two week period of observation. The guinea pig that was inoculated with the Group E streptococcus culture showed no observable symptoms of disease during a 21 day postinoculation period and revealed no lesions at autopsy. The test organism was not recovered from cervical region lymph nodes, heart's blood, and kidney.

Discussion of Results

In Experiment A, throat abscesses, indistinguishable in appearance from those seen in field cases, were induced in pigs that were fed a pure culture of the Group E streptococcus, and that organism was isolated in pure culture from the exudate of these lesions. It was determined as a result of the cultural survey of swine throat abscesses reported in the preceding chapter, that the Group E streptococcus is regularly associated with the exudate of these lesions and that other kinds of bacteria are not regularly associated with them. These facts prove that the Group E streptococcus is an etiologic agent of the swine throat abscess disease and, furthermore, indicate that it is the principal etiologic agent of that condition.

The failure of abscesses to develop in occasional animals (such as RG53) that were fed the Group E streptococcus was not explained. The findings in Experiment A did reveal that the etiologic agent is quite invasive, when fed to susceptible swine, and that it does not seem to require trauma or any other stress factor to enable it to invade and
establish an infection of the cervical region lymph nodes. Following that invasion certain symptoms were observed in the infected animals that have not been reported in field cases of the throat abscess disease. At least 36 hours postinoculation there is an acute febrile stage accompanied by leucocytosis, anorexia, and depression. The failure to note these symptoms in field cases is understandable in view of the transient nature of the anorexia and depression and the fact that veterinary practitioners would have no reason for taking rectal temperature readings or making leucocyte counts of apparently normal animals. The general thriftiness observed in the diseased pigs, even those affected with multiple, large abscesses, correlates with similar observations in uncomplicated field cases.

The pathogenesis of the lesions was indicated. Apparently the invading organism establishes a focus or foci of infection in the affected lymph node, and the host attempts to encapsulate and localize the infection. There seems to be a progressive enlargement of the abscess(es) at the expense of the surrounding lymphoid tissue until, finally, all lymphoid tissue is obliterated and the thickened, distended capsule becomes the wall of a large, single cavity abscess. Apparently the internal pressure of the lesions causes those that are superficially located to migrate to the adjacent skin surface, pressure necrosis of the skin overlying the lesion occurs, and eventually the fragile, necrotic tissues covering the abscess rupture and permit the exudate to drain.

The minimal period of about 3 weeks from the time of inoculation until the development of visible lesions coincides with the interval
reported by Snoeyenbos (20) between the introduction of normal pigs into infected premises and the appearance of visible lesions.

No explanation has been advanced to account for the spread of the Group E streptococcus among animals within an infected herd. The fact that abscesses can develop and drain within a period of 5 to 7 weeks suggests that one or two pigs affected with throat abscesses at weaning time could supply the inoculum (on drainage of those lesions) for the oral inoculation of pen mates in an ever-widening vicious cycle that would account for a high percentage of diseased animals in that herd at market time. As a matter of fact, Collier (6) reported throat abscesses in pigs at 6 and 8 weeks of age in a herd where the disease was enzootic. A pig with a grossly-observable abscess at 6 weeks of age must have picked up the organism from its environment at least 3 weeks previously. What is/are the source(s) of infection of young pigs that do not come in contact with abscess exudates?

The occurrence of the disease in animals so young causes one to consider the dam as a probable source of the infection. In one herd, where the throat abscess disease was enzootic, Collier (7) isolated the Group E streptococcus from the nasal mucus, tonsillar tissue, maxillary sinus, and mammary gland of apparently normal sows at slaughter. The report of Hare (11) supports the association of the Group E streptococcus with the porcine mammary gland.

The lack of abscess development in either of the pigs in the dirty pen contact trial of Experiment E may indicate 1) that the Group E streptococcus does not persist in soil, or 2) that the animals picked up
and the general feature to evidence parenchymally elsewhere in the
marked preservation of that organ since in the central region lymph node
however, the intradermal injection done in cream was not electrocautered, the
resultant when much larger doses of injection were given in the cream,
interpharyngeal injection of the carcinoma were comparable to those
of the oesophageal symptoms and lesions that developed following intramuscular
Group B streptococcus to the max—pharynx and buccal cavity of mice.
The results of experiment G narrowed the portal of entry of the

Injection for the normal eyes.
the latter superscription were correct, then perhaps there was no source of
that the dermal amount was not shedding the group B streptococcus. If
interpreted (1) that both animals were resistant to the injection, or 2)
contact with an external source (this un像是 allowed) absence may be
The lack of abscess development in either of the pigs placed in

During the contact period
would indicate that there were viable cells of that organism in the pen
normal mesodermal lymph node of one of the contact animals, however,

In that pen, the recovery of the group B streptococcus from ear aspiration
without the previous organisms had developed abscesses, no abscesses drained
occupyance of that pen 2 days before the contact trial started and that,

It should be borne in mind that the inoculation was done to the prelous
streptococcus may not persist longer than a few days in pen surroundings.
the read trough and pen trial in experiment G suggest that the group B
 pigs used were resistant to the injection. The results of culture
or observation was too short for abscesses to develop, or 2) that the period
less than a minimal infective dose of that organism, or 3) that the period
was a phenomenon common to all of the routes used. The interruption of postinoculation parameters that could not withstand or sustain eternally routes the test organism encountered barriers and
intercepted. Interception
then it must enter through the lymph channels when these nodes do become the central region lymph nodes by way of the blood circulation system. If the group of streptococci cannot directly at the end of that intestinal. If the group of streptococci cannot directly postinoculation, because the organism was recovered from sterilized blood of the various central region lymph nodes during the first 2 hours
numbers of the lice bacteriae were intercepted through the capillaries
interception a large dose of the group of streptococci could have been recovered from the blood stream. Safety
the organism to survive in the blood stream.
2) apparent immunity of the organism to escape from the blood stream,
coocccus inoculum demonstrated (1) lack of lethal effect by the organism,
(2) important effect of the organism to reach those nodes, these and many similar
questions remain unresolved.
pathogenic effect after all these nodes, these and many similar

Any of the continuous lymph nodes, or were the organism unable to exact any
inoculation method of so gradual and complete that no organsisms reached
inoculations selected subsequently in the body of the rabbit were that large.

in the central region lymph nodes but not in other lymph nodes of the body of the rabbit. What happened in the case of the 10 ml. of broth cutaneous
in the group 2 streptococci to gain entry to and exact a pathogenic effect
what is the peculiar mechanism or set of circumstances that enables
entrusted body emphasized the high degree of specialization.
period before the onset of pyrexia varied in length depending on the route used. Apparently there is a pyrogenic substance associated with the bacterial cell or its metabolites that induces the pyrexia. That substance is formalin-inactivated, because formalin-inactivated whole-culture bacterin injected into animals failed to elicit a febrile reaction.

There were no clear-cut indications of the value of bacterin as a prophylactic agent in the throat abscess disease. The pattern of post-challenge abscess production was confusing because 2 of the unvaccinated controls failed to develop lesions. The pen of animals that received bacterin intravenously developed the higher postvaccinal level of anti-Group E streptococcal agglutinins (Fig. 2), yet each of them developed abscesses following challenge with the live organism. Obviously there was no correlation in that case between the level of agglutinins and resistance to the disease. The test organism was quite regularly recovered from nasal mucus and tonsillar tissue of the various animals regardless of whether or not abscesses developed. Does that finding mean, in event bacterin prophylaxis of the throat abscess disease does prove effective, that the infected carrier state may persist in vaccinates exposed to the live organism? Additional experimental work is needed to evaluate bacterin prophylaxis of throat abscesses. At the same time it would be very pertinent to determine whether or not a high percentage of the vaccinates do carry the Group E streptococcus as an inapparent infection following challenge with the living organism.

On the basis of results obtained in the survey of swine throat abscesses reported in the preceding chapter it was reasoned that Coryne.
pyogenes and \textit{Past. multocida} could not be considered principal etiologic agents of that condition, because of their relatively low overall incidence. The inability of either of them to induce abscesses when fed in a massive inoculum to normal swine in Experiment E indicates that they lack the invasive property associated with the Group E streptococcus under the same conditions. In other words, it seems doubtful that either \textit{Coryn. pyogenes} or \textit{Past. multocida} have the ability to penetrate the intact mucosa of the naso-pharynx and establish infections of the cervical region lymph nodes. When those two kinds of organisms were combined with the Group E streptococcus and fed as a mixed inoculum to swine, the result was no different than if only the streptococcus had been fed. That finding indicates that neither \textit{Coryn. pyogenes} or \textit{Past. multocida} reach the site of abscess formation in normal animals by accompanying the Group E streptococcus as it invades.

What, then, are the unusual conditions under which either of those organisms may become established in the exudate of a swine throat abscess? Collier (7) found an association between symptoms of respiratory tract disease, accompanied by marked edema of the sub-mandibular region of swine, and the recovery of both \textit{Past. multocida} and the Group E streptococcus from the exudate of early stage throat abscesses in those animals.

The frequent association of \textit{Coryn. pyogenes} with the exudate of abscesses having necrotic overlying skin has already been mentioned. That organism is frequently associated with infections predisposed by trauma of the skin of swine. Perhaps trauma of skin caused by the pressure of a developing throat abscess (in which the Group E streptococcus is the etiologic agent) enables \textit{Coryn. pyogenes} to infect and penetrate the
devitalized or necrotic tissue overlying the lesion and thus gain access
to the exudate contained within its capsule.

The marked in-vitro sensitivity of the Group E streptococcus to
penicillin suggested testing that antibiotic in swine as a therapeutic
agent against that organism. In the animals of Experiment F, that were
treated with penicillin at 72 hours postinoculation, the shortening of
the febrile period indicated sensitivity of the organism in-vivo. The
fact that no evidence of abscess development was found in three animals
in which treatment was delayed until 16 days postinoculation and that there
were no untreated controls, however, leaves room for reasonable doubt that
abscesses would have developed in some of the animals, even without penicil-
lin therapy. Further evaluation of penicillin as a therapeutic agent in
swine infected with the Group E streptococcus is indicated.

The results of inoculating the Group E streptococcus into small
laboratory animals supports the belief that it is an organism of limited
host range. Mice inoculated intraperitoneally with culture of that organism
were apparently unaffected by relatively large doses. In rabbits inoculated
intravenously the indications were that the M.L.D. of the test organism
for that animal is relatively large. The Group E streptococcus evidenced
pathogenicity on intradermal inoculation of the rabbit, however, the skin
abscesses were confined to the sites of inoculation, and the test organism
did not survive in the exudate of those lesions. The guinea pig was found
to be insusceptible to either oral or subcutaneous inoculation of that
organism.
SUMMARY AND CONCLUSIONS

1. A cultural survey of the exudates of 492 swine throat abscesses revealed Lancefield's Group E streptococcus to be associated with 85.6 per cent of those specimens.

2. The incidence of that organism from specimens obtained from Ohio, Illinois, and Minnesota was quite similar to that of specimens obtained in Iowa.

3. Other kinds of bacteria recovered from some specimens were believed to be secondary, because of 1) the low overall incidence of each kind, 2) the high overall incidence (67.3 per cent) of pure cultures of the Group E streptococcus, and 3) because of the frequent occurrence of the other kinds of bacteria in mixtures with the latter organism.

4. Throat abscesses were regularly induced in young swine given Group E streptococcus culture in the feed. Intranasal or intrapharyngeal inoculation of that organism produced identical results, however, inoculation by various other routes failed to induce throat abscesses.

5. Pyrexia, leucocytosis, anorexia, and depression were clinical findings associated with the early stages of the throat abscess disease.

6. The cervical region lymph nodes were found to be the sites of abscess formation.

7. The feeding of Corynebacterium pyogenes or Pasteurella multocida culture to swine did not result in throat abscess formation.
8. On the basis of findings summarized above, it was concluded that 1) Lancefield's Group E streptococcus is the principal etiologic agent of the swine throat abscess disease and that 2) it is an organism of limited general pathogenic ability with a marked predilection for the naso-pharynx and cervical region lymph nodes of swine.
LITERATURE CITED


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