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ENTERIC *STREPTOCOCCUS DURANS*--AN ADHERING STREPTOCOCCUS AS A CAUSE OF DIARRHEA IN SUCKLING PIGLETS?

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ENTERIC STREPTOCOCCUS DURANS--AN ADHERING STREPTOCOCCUS
AS A CAUSE OF DIARRHEA IN SUCKLING PIGLETS?

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INTRODUCTION

Streptococci have been associated with numerous and varied disease
syndromes in animals, including septicemia, pneumonia, lymphadenitis,
mastitis, metritis, arthritis, vegetative endocarditis, and wound
infections (15, 18). Conversely, many of the streptococci, particularly
the alpha hemolytic and non-hemolytic types, have been considered normal
flora or opportunistic bacteria of less consequence than beta hemolytic
groups; hence much of the clinical and experimental interest has focused
on beta hemolytic streptococci (6, 9, 12).

Group D streptococci have been divided into two groups, namely
enterococci and non-enterococci. Enterococci belonging to this group
have been regarded as normal or possibly beneficial flora in the upper
gastrointestinal tract (1, 7, 10, 17). Pathologic changes in microvilli
were alluded to in areas of adherence in chicken duodenum colonized by
Streptococcus faecium SY1 (10).

Adherence of streptococci to gastrointestinal mucosal surfaces is
an infrequent finding in animals, but has been reported to occur in pig
stomach and intestine (1, 7, 17), and in crop and duodenum of chickens
(10). Adherence of various groups of streptococci has also been
reported to occur in pharynx, urinary tract, and vagina of humans (9,
12). Fibrillar projections from streptococci are considered essential
for adherence of the organism to mucosal surfaces. Fibrils arise from
the cell wall and consist at least partly of M protein covered with
lipoteichoic acid (9, 12, 16). Pathogenicity has been associated with
the presence of M protein.

NATURAL DISEASE

Nineteen cases of enteritis primarily or secondarily associated
with streptococci have been submitted to the South Dakota Veterinary
Diagnostic Laboratory from 1978 to the present time. Of those 19 cases,
13 have had other enteric pathogens identified concurrent with
streptococcal infection, while the remaining 6 cases had no other
identifiable enteric pathogens (Table 2). TGE, rotavirus, adenovirus,
enterovirus, and E. coli were identified along with streptococci in 2,
7, 1, 1, and 5 cases, respectively. Affected pigs, submitted from South
Dakota, Minnesota, Iowa, Nebraska, and Kansas, varied from 3-17 days of
age. Streptococci were cultured from intestine or intestinal swabs from
14 cases and streptococci were adhering to mucosa in 17 cases.
Sensitivities were done on 6 isolates from pigs with the following
results: 6 were sensitive to trimethoprim/sulfa, 2 to ampicillin, 2 to
novobiocin, 1 to tylosin, and 1 to erythromycin (Table 3).

Three cases of calf enteritis with streptococcal adherence to
intestinal mucosa have been identified (Table 2). Two cases were
submitted in 1983 and 1 in 1984. Alpha streptococci were cultured from
only 1 of the 3 cases, and it was untypable. The isolated streptococcus
was sensitive to ampicillin, penicillin, gentamicin, spectinomycin,
vetisulid, and furox (Table 3). Heavy infestations of cryptosporidia
were present in 2 of the 3 calves, and the remaining calf did not have

other identifiable enteric pathogens. Affected calves ranged in age from 7-60 days. The two calves affected with cryptosporidia were 7-10 days of age, and the 60-day-old calf did not have other identifiable pathogens. Streptococci adhered to sections of intestine not affected with cryptosporidia. All three calves were from central and south central South Dakota.

<u>EXPERIMENTALLY PRODUCED DISEASE</u>

<u>MATERIALS AND METHODS</u>

<u>Organism</u>--An alpha hemolytic streptococcus characterized as <u>Streptococcus durans</u> was isolated from small intestine of a 5-day-old pig in which gram positive cocci were adhering to mucosa. The organism was cultured on sheep blood agar plates for 18 hours at 37°C, and colonies rinsed from the agar with brain heart infusion broth. One ml of the suspension contained approximately 2×10^9 organisms.

<u>Animals</u>--Gnotobiotic pigs were derived by cesarean section and housed in gnotobiotic units capable of holding 4 pigs. One ml of the inoculum described above was given orally to 14, 3 to 14-day-old gnotobiotic pigs. One non-inoculated pig served as a control (Table 1).

Two conventional 10-day-old suckling pigs orally inoculated with the same culture and by the same method as the above gnotobiotic pigs, were left with the sow and observed 14 days. One uninoculated pig served as a control.

<u>Collection of Specimens</u>--Segments from nine sites of the small and large intestine were collected for light microscopy (LM) and transmission electron microscopy (TEM). The first segment of small intestine (SI) was removed from the duodenum near the stomach and the last segment from terminal ileum near the ileocecal valve. Remaining segments were collected at 1/6, 1/3, 1/2, and 2/3 the distance from the pylorus to the ileocecal junction. Segments were designated duodenum, upper jejunum, lower jejunum, midportion of small intestine, upper ileum, and lower ileum. Segments of large intestine were removed from cecum, apex of spiral colon, and terminal colon.

<u>Light Microscopy</u>--Segments of intestine were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin. Selected sections were also gram stained.

<u>Transmission Electron Microscopy</u>--Sections of intestine as listed above were fixed in Karnovsky's fixative, post-fixed in 1% osmium tetroxide, dehydrated through acetone, and embedded in epon resin. Intestinal segments from selected pigs were also fixed in cacodylate buffered glutaraldehyde containing ruthenium red using previously described procedures (5, 8) and processed as above. Survey sections 1-2 μ were cut and stained with 1% toluidine blue. Thin sections were placed on 300 mesh copper grids, stained with uranyl acetate and lead citrate, and viewed with a Hitachi Hu-12 TEM (Hitachi, Ltd., Tokyo, Japan).

<u>Gut Loops</u>--Duplicate gut loops were done in 2, 9-day-old conventional pigs. Four <u>S. durans</u> field isolates were each inoculated into duplicate gut loops in those 2 pigs. The inoculums consisted of 1 ml of an 18-hour brain heart infusion broth culture containing approximately 2×10^9 organisms. Alternate loops were uninoculated

controls. Positive and negative enterotoxigenic E. coli control loops
were included in each pig. Pigs were killed 8 and 10 hours
post-inoculation (PI). Gross observations were recorded and tissues
were collected for LM.

Bacterial Examination--Segments of jejunum and ileum were collected
for bacterial examinations. Each segment was cultured on blood agar,
tergitol-7, and brilliant green agar. The cultures were incubated 24
hours at 37°C and examined for bacterial growth.

Negative Stain Electron Microscopy--Cecal content was collected
from each pig, processed, and examined for viruses using previously
described procedures (11).

Fluorescent Antibody Tests--Segments of jejunum and ileum were
examined for viruses using frozen sections stained with
anti-transmissible gastroenteritis virus and anti-rotavirus conjugates
(11).

RESULTS

A summary of findings from field cases submitted to SDADR&DL is
reported in Table 2.

Four streptococcus cultures isolated from porcine enteritis field
cases were submitted to Dr. Richard Facklam, Communicable Disease
Center, Atlanta, GA, who characterized them serologically and
biochemically as Streptococcus durans.

Clinically, all inoculated gnotobiotic pigs developed watery to
mucoid yellow diarrhea within 24 hours of inoculation. Decreased
appetite and loss of body condition accompanied diarrhea. Pigs also
developed rough hair coats. None of the pigs died; however, diarrhea
continued throughout the two week experimental period.

Microscopically, all of the inoculated gnotobiotic pigs had
adherence of streptococci to mucosal epithelial cells of both small and
large intestine. Adherence occurred diffusely in SI from upper jejunum
through ileum on PI days 1 and 2. Adherence became more focal and
numbers of adhering bacteria were reduced on day 3 and following.
Streptococcal adherence was focal in large intestine, but occurred in
all areas examined.

Light microscopic lesions in small intestine consisted of necrosis,
cuboidal to squamous metaplasia, vacuolation, and piling of villous
epithelium. Villous atrophy and fusion became apparent in jejunum and
ileum by PI day 2 and was moderately severe by PI days 3. Regeneration
of villous length was evident by PI day 11, however, streptococci in
reduced numbers were still present on mucosa and in intestinal lumens.
Lamina propria of intestine initially was loosely arranged as if
edematous, and central lacteals were dilated. Polymorphonuclear cells
and mononuclear cells were in lamina propria and migrating through
mucosa.

Colon had focal adherence of streptococci to mucosa throughout the
two week experimental period. Number of streptococci adhering was
reduced on PI day 5 and following. Epithelium over some mucosal ridges
was necrotic and separating from underlying basement membranes.
Necrosis and nuclear debris were evident in lamina propria beneath
mucosa of colonic ridges.

Streptococci were observed adhering to sloughed necrotic

epithelial cells in stomach, but not to intact gastric epithelium.

Duplicate gut loops in conventional pigs inoculated with four field isolates of Streptococcus durans did not show enlargement or fluid accumulation. All four isolates of streptococci adhered to intestinal mucosa upon examination microscopically.

TEM revealed fine fibrillar projections extending in all directions from streptococci. Some extended to and terminated on mucosa, while others extended between organisms. A clear zone surrounded the organisms.

Two, 10-day-old orally inoculated conventional pigs which remained on the sow and continued nursing showed no signs of diarrhea or other clinical evidence of disease throughout a 2 week observation period.

Bacterial and viral postmortem examinations of gnotobiotic pigs revealed no pathogens other than Streptococcus durans. Control pigs remained normal clinically, culturally, and histologically throughout the experiment.

DISCUSSION

Observation of streptococcal colonization of pig intestine from field cases of enteritis indicated streptococci may cause or complicate baby pig enteritis.

Preliminary pathogenicity studies with this strain of Streptococcus durans suggests it may be a primary enteric pathogen which has the ability to colonize intestine and produce diarrhea in young gnotobiotic pigs. Diarrhea occurs simultaneously with colonization of intestinal mucosa, necrosis of villous epithelium, villous atrophy, and villous fusion. Diarrhea was of moderate severity, and when uncomplicated by other intestinal pathogens, was non-fatal, but persistent. Although colonization of gastrointestinal tract has been reported with fecal streptococci, there has been little evidence presented to indicate their capacity to cause enteritis or pathological changes in intestine (1, 7, 16, 17). In general, gram positive cocci have not been considered intestinal pathogens.

Although streptococci colonize intestine in a manner similar to E. coli, the mechanism by which they produce diarrhea appears to be different (4, 13). Streptococcus durans causes necrosis of villous epithelium and villous atrophy similar to that observed with viral and coccidial enteritis. It did not produce secretory diarrhea in gut loops of conventional pigs such as that produced by enterotoxigenic E. coli. The basic cause of necrosis of villous epithelium was not apparent from present experiments; however, streptococci produce a number of toxins which are capable of damaging cells, or Streptococcus durans may cause direct mechanical damage to villous epithelium. Further experimentation needs to be directed to this problem. Other agents that cause villous atrophy, such as coronavirus, rotavirus, and coccidia, are reported to cause malabsorption and maldigestion that result in diarrhea. This may also apply to enteritis caused by Streptococcus durans.

Fimbriae observed by TEM in our study extend to mucosa and between adjacent bacteria. Fimbriae covered with lipoteichoic acid (LTA) which project from the cell wall of group A streptococci are thought to be associated with ability to adhere to mucosal surfaces (2, 3, 14).

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Fimbriae of group A streptococci originate from the protein layer of the cell wall and project through the capsule. They are composed at least partly of M protein covered with lipoteichoic acid (9). Fimbriae from group D streptococci have not been characterized. The lucent zone around the organism is reported to represent capsular material (10).

To the present time, we have been unable to reproduce enteritis in conventional pigs by oral inoculation with Streptococcus durans. That effort continues.

In conclusion, Streptococcus durans has been considered a normal part of the intestinal flora. We would contest that assumption in light of evidence from field case submissions and because of its ability to colonize itnestinal mucosa and create pathologic changes in intestine of gnotobiotic pigs.

ACKNOWLEDGMENTS

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TABLE 1.   Experimental gnotobiotic pigs

Litter	No. of principles	No. of controls	Age when inoculated[a]	Age when killed[b]
1	6		3	24, 48, 120, 192, 264, 336
		1	Not infected	10 days
2	2		2	2 weeks
	4		6	24, 48, 72, 96
	4		14	24, 72, 96, 120

[a] Days.
[b] Hours, unless noted.

TABLE 2. Summary of streptococcal enteritis field cases submitted to the South Dakota Animal
         Disease Research and Diagnostic Laboratory

	Total cases	Age	States	Culture	Adherence	Rota	TGE	E. coli	Adeno	Entero
Pigs	19	3-17 days	IA, NE, SD, KS, MN	14	17	7	2	5	1	1
Calves	3	7-60 days	SD	1	3 (2 of the 3 had cryptosporidiosis also)					

TABLE 3. Sensitivity patterns of Streptococcus durans isolated from field cases

	Total	Trimethoprim/sulfa	Ampicillin	Novobiocin	Tylosin	Erythromycin
Pigs	6	6	2	2	1	1
Calves	1	Ampicillin, gentamicin, penicillin, spectinomycin, vetisulid, and furox.				