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Characterization of Predominant Bacteria from the Colons of Normal and Dysenteric Pigs

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Bacterial populations adherent to the mucosa of the proximal colons of weaned, healthy pigs were compared with populations from pigs with dysentery induced by inoculation with a culture of *Treponema hyodysenteriae*. Isolates (136) representative of the predominant flora adherent to colonic epithelia of normal pigs and isolates (162) from pigs with dysentery were cultured anaerobically on a rumen fluid-based medium and characterized. Most (71%) of the isolates from colonic epithelia of normal pigs were gram positive, whereas 88% of the epithelia-associated isolates from pigs with dysentery were gram negative. The geometric mean of colony counts was $5.7 \times 10^7/cm^2$ of colonic tissue from three normal pigs and $7.7 \times 10^8/cm^2$ from four pigs with dysentery. A number of isolates obtained from contents of the lumens of normal pigs and pigs with dysentery were also characterized. Comparison of isolates from epithelial tissue and from contents of the lumens of the same pig indicated that these populations were different. Our results indicate that physiological changes that occur in the colons of pigs with dysentery are accompanied by marked changes in the microbial populations in the colons. The factors which regulate the population changes are not yet understood.

Bacterial adherence to surfaces in many natural environments is well known, but remarkably little is known about bacterial populations attached to the colonic epithelia of swine or of the physiological factors which regulate the selection of either indigenous or pathogenic bacterial populations. Although a number of studies have examined bacteria isolated from intestinal or fecal material of swine (1, 4, 7, 14, 16, 18, 19, 21, 22, 27) and the effects of antibiotics or other drugs on intestinal populations (6, 8, 24), little is known of the changes in such populations associated with diarrheal diseases (2, 16, 25).

The present study was conducted to examine microbial populations of pig colons and to characterize any changes in such populations associated with swine dysentery. This disease is a naturally occurring mucohemorrhagic diarrheal disease of swine that occurs throughout the world. The primary lesion of this disease is inflammation and superficial necrosis of the mucosa of the large intestine with no apparent involvement of the small intestine. Typical signs of swine dysentery can be induced experimentally in swine by oral inoculation with *Treponema hyodysenteriae* (28). In the present study, we describe the predominant bacteria (other than *T. hyodysenteriae*) that inhabit the colonic lumen and mucosal epithelium of both normal pigs and pigs with experimentally induced swine dysentery.

MATERIALS AND METHODS

Samples from pigs. Colonic samples were obtained from weaned, crossbred pigs (age, 7 to 9 weeks) obtained from the specific pathogen-free herd at the National Animal Disease Center. These pigs were fed a standard diet, designed and mixed at the National Animal Disease Center, that contained 15% protein, 12% fiber, 2.5% fat, and a complete vitamin and mineral premix but no antibiotics. Four pigs became infected after intragastric inoculation on 2 successive days with 100 ml of broth cultures (24 to 36 h) of *T. hyodysenteriae* B204 that contained $\geq 10^7$ viable cells per ml. *T. hyodysenteriae* was grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) containing 0.25% glucose and 10% bovine fetal serum (15). Inoculated pigs were killed, and necropsies were performed 2 to 3 days after the onset of diarrhea (within 12 days after challenge). Samples were also obtained from three uninoculated pigs from the same herd.

Processing of the samples. Both ends of a 20- to 30-cm segment of spiral colon were ligated, and the segment was removed by aseptic procedures. The segment was opened and tacked, mucosal side up, to a board covered with sterile aluminum foil.

Phosphate-buffered saline (500 ml) was poured over the tissue to rinse off colonic contents, and then five disks (diameter, 12 mm) were cut and placed in 99 ml of anaerobic dilution solution (5). These steps were performed rapidly so that cecal tissue was exposed to the saline rinse and to room air for less than 5 min. Disks were rinsed by shaking the suspension 10 times. The disks were then removed with forceps, placed in a second 99-ml volume of anaerobic dilution solution, and blended for 2 min in a Waring blender flushed with CO_2. Serial dilutions and inoculations were made by the anaerobic technique introduced by Hungate (13).

Colonic contents were placed in a beaker and mixed by stirring, and an 11-g sample of the material was blended (Waring blender) with 99 ml of an anaerobic dilution solution for 1 min under a CO_2 atmosphere. Serial 10-fold dilutions in the anaerobic dilution fluid and inoculations were made as described above.

Culture media and methods. Bacteria were enumerated and isolated with medium MCCA, which was a modification of medium CCA (3), in that endogenous fermentable, energy-yielding substrates were not depleted from the rumen fluid and agar. MCCA medium contained 40% rumen fluid plus the following (in milligrams per milliliter): cellobiose, glucose, maltose, starch, and xylose (each 0.25); Trypticase (2.0); glycerol (1.25); hemin (0.125); agar (1.5%), cysteine hydrochloride (0.25); and minerals.

The methods and media used for culture and determination of physiological properties of strains have been described previously (19). Some isolates were presumptively

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TABLE 1. Effects of washing colonic tissue disks or the removal of adherent and nonadherent bacteria

Animal no.	Culture counts (CFU × 10^{-7}/cm² of tissue) in:								
	Sample A		Sample B						
					Epithelium-adherent bacteria (%) in[c]:				
	Rinse no. 1	Epithelium-adherent bacteria[a]	Rinse no. 1	Epithelium-adherent bacteria[b]	Rinse no. 2	Rinse no. 3	Rinse no. 4	Rinse no. 5	Tissue (blended)
1	21.0	17.0	22.8	21.8	23.4	20.2	2.2	0.8	53.2
2	6.7	22.3	8.8	22.5	20.4	10.2	6.7	6.2	56.4
3	4.2	7.2	1.2	4.6	21.7	11.7	7.6	6.1	52.2

[a] Epithelium-adherent bacteria (sample A) refers to culturable bacteria recovered from tissues after removal of organisms by rinse 1.
[b] Epithelium-adherent bacteria (sample B) includes bacteria recovered in rinses 2 through 5, plus blended tissue after rinses, expressed as CFU (× 10^{-7}) per cm² of tissue.
[c] Percent epithelium-adherent bacteria in rinses 2 through 5 and in blended tissue after rinses.

identified to the genus level or were unassigned because they did not fit well into established groups. *T. hyodysenteriae* was recovered from the four pigs with dysentery and identified by culture (26) and dark-field examination. Ammonia was determined colorimetrically by the indophenol blue reaction (10). The Burke method (9) for gram staining was used, and hemolytic activity was determined by the use of plates of brain heart infusion agar (BBL Microbiology Systems) supplemented with 5% defibrinated sheep blood that were incubated in anaerobic jars containing CO_2.

Antigens from representative strains of streptococci were prepared as described previously (23) from strains grown on modified peptone yeast glucose (MPYG) broth (19). All strains were tested for group-specific antigens (Lancefield groups A, B, C, D, E, F, G, H, K, L, M, N, O, P, Q, T, U) by an agarose gel slide double-diffusion precipitin test (23). Strains were also tested for group-specific antigens (groups A, B, C, F, G) with a Sero-STAT Streptococcus latex test kit (Scott Laboratories, Inc., Fiskeville, R.I.).

Adherent bacteria. In this study, we considered bacteria not removed by the first rinse of epithelial tissue disks as populations that adhered to epithelial tissue. This designation was based on the results of experiments conducted to determine, by culture counts, the patterns of release of bacteria with successive rinses from the tissue disks. From each of three pigs, two separate sets (five 12-mm diameter disks) of colonic tissue were obtained and rinsed as described above. One set was then similarly rinsed four more times before the tissues were blended. The other set of disks was blended without additional rinses. Culture counts of this rinse indicated that 31.5 ± 15.6% of the total population recovered from the tissue was freed from the tissue (samples A and B) during the first rinse. A progressive release of attached bacteria occurred with successive rinses. Although the pattern of release differed among samples from different pigs, more than one-half of the population remaining after the first rinse was still associated with tissue disks after five rinses (Table 1). The effect of the brief exposure of the tissue to air and the aerobic rinsing solution on recovery of viable bacteria or the extent of loss of the mucous layer with its bacterial population is not known.

RESULTS

The mean (± standard error) of log colony counts of bacteria from colonic epithelia was 7.76 ± 0.36/cm² for samples from three normal pigs and 8.89 ± 0.31/cm² for samples from four pigs with dysentery. A total of 136 isolates from colonic epithelia of normal pigs and 162 isolates from pigs with dysentery were characterized. In addition, 83 and 87 isolates from the colonic lumen contents of normal pigs and pigs with dysentery, respectively, were characterized.

Normal pigs and epithelial bacteria. The predominant culturable bacteria recovered from colonic epithelia from normal pigs are listed in Table 2. Gram-positive bacteria constituted over 71% of the flora and included *Streptococcus* sp. group U-2 (54.5%), *Lactobacillus acidophilus* (8.1%); *Lactobacillus fermentus* (3.7%); *Bifidobacterium adolescentis* (1.5%); and *Coprococcus* sp. group A-1, *Eubacterium aerofaciens*, *Peptococcus asaccharolyticus*, and *Peptostreptococcus productus* (less than 1% each). Gram-negative organisms comprised 29% of the epithelial isolates. These included *Bacteroides* sp. group A-1 (18.4%), *Selenomonas ruminantium* (4.4%), *Fusobacterium prausnitzii* (2.2%), *Gemmiger formicilis* (1.5%), and *Escherichia coli* and *Leptotrichia buccalis* (less than 1% each). Bacteria listed as *Bacteroides* sp. group A-1, *Selenomonas ruminantium*, and *Streptococcus* sp. group U-2 were recovered from epithelial samples from all three of these pigs, whereas nine others (Table 2) were represented by isolates from only one of the pigs.

Dysenteric pigs and epithelial bacteria. The distribution of bacterial groups adhering to the colonic epithelium of four

TABLE 2. Distribution of bacteria adherent to colonic epithelia of normal pigs

Bacteria[a]	No. of isolates from pig no.:			% of total[b]
	1	2	3	
Streptococcus sp. group U-2	6	37	31	54.4
Bacteroides sp. group A-1	5	7	5	12.5
Lactobacillus acidophilus	9	0	2	8.1
Bacteroides ruminicola	0	2	4	4.4
Selenomonas ruminantium	3	1	2	4.4
Lactobacillus fermentus	5	0	0	3.7
Fusobacterium prausnitzii	0	1	2	2.2
Bacteroides amylophilus	0	2	0	1.5
Bifidobacterium adolescentis	1	0	1	1.5
Gemmiger formicilis	0	0	2	1.5
Eubacterium aerofaciens	1	1	0	1.5
Anaerovibrio lipolytica	0	0	1	0.7
Coprococcus sp. strain A-1	1	0	0	0.7
Escherichia coli	0	0	1	0.7
Leptotrichia buccalis	0	0	1	0.7
Peptococcus asaccharolyticus	0	1	0	0.7
Peptostreptococcus productus	0	1	0	0.7

[a] A total of 136 isolates were tested.
[b] Three pigs were tested.

TABLE 3. Distribution of bacteria adherent to colonic epithelia of pigs with dysentery

Bacteria[a]	No. of isolates from pig no.:				% of total[b]
	4	5	6	7	
Acetivibrio ethanolgignens	19	13	10	3	27.8
Selenomonas ruminantium	6	5	12	2	15.4
Escherichia coli	3	18	2	0	14.2
Eubacterium sp. group A-1	0	1	5	8	8.6
Fusobacterium sp. group A-1	0	0	9	3	6.8
Streptococcus sp. group U-2	10	0	0	0	6.2
Clostridium putrificum	0	7	0	0	4.3
Fusobacterium necrophorum	0	0	1	4	3.1
Bacteroides sp. group B-2	5	0	0	0	3.1
Anaerovibrio lipolytica	0	0	0	4	2.5
Bacteroides ruminicola	0	1	0	3	2.5
Peptococcus saccharolyticus	0	0	3	0	1.9
Bacteroides asaccharolyticus	0	0	0	1	0.6
Bacteroides multiacidus	0	0	0	1	0.6
Desulfomonas pigra	0	0	0	1	0.6
Leptotrichia buccalis	0	0	1	0	0.6
Peptostreptococcus productus	1	0	0	0	0.6

[a] A total of 162 isolates were tested.
[b] Four pigs were tested.

pigs with dysentery is given in Table 3. The majority (79.4%) of organisms isolated were gram-negative bacteria, and the most numerous groups were *Acetivibrio ethanolgignens* (27.8%), *Selenomonas ruminantium* (15.4%), *Escherichia coli* (14.2%), and *Fusobacterium* sp. group A-1 (6.8%). The remaining gram-negative bacterial groups, including *Bacteroides* sp. group B (3.1%), *Anaerovibrio lipolytica* (2.5%), *Bacteroides ruminicola* (2.5%), *Bacteroides asaccharolyticus* (1.9%), *Bacteroides multiacidus* (0.6%), and *Desulfomonas pigra* (0.6%), were minor constituents of the epithelia flora from pigs with dysentery.

Gram-positive bacteria constituted 20.6% of the isolates from colonic epithelium of pigs with dysentery. These included *Eubacterium* sp. group A-1 (8.6%), *Streptococcus* sp. group U-2 (6.8%), *Clostridium putrificum* (4.3%), *Peptococcus saccharolyticus* (1.9%), and *Peptostreptococcus productus* (0.6%).

Of the 350 epithelial isolates obtained on primary isolation from both normal and dysenteric pigs, 52 were subsequently lost. Two hundred ninety-eight isolates were assigned to 27 genera. Only seven bacterial groups were present in both normal and dysenteric pigs. It is evident that bacterial populations adherent to the colonic mucosa of pigs with dysentery differed significantly from those of normal pigs.

Colonic lumen bacteria. Data in Table 4 allow for the comparison between epithelial and luminal bacterial populations from individual pigs. Epithelial samples from both of the normal pigs contained higher proportions of streptococci than were found in luminal populations, whereas *Fusobacteria* spp. tended to be more abundant in the lumen. With other bacterial groups, differences between luminal and wall populations were not consistent.

With the dysenteric pigs, *Escherichia coli* and *Clostridium putrificum* were major bacteria identified from the epithelial samples of one of these pigs but were not isolated from lumen samples from the same pig, and they were not isolated from either epithelial or lumen samples of the other infected pig. Organisms grouped in the genus *Bacteroides* were a much larger proportion of the luminal population than of the epithelial population in both infected pigs. Thus, although differences between colonic epithelial and luminal populations from the same pig have been found, there were also large animal-to-animal differences, and additional studies are needed before predictions can be made concerning the differences between epithelial and luminal populations in either normal or infected animals.

Bacterial groups that did not fit with previously described species. Characteristics of isolates not readily placed into previously described species are presented in Table 5. Some strains were similar to previously described species, but as they differed in a number of important properties, they were assigned to the genus level. We assigned letter designations within the appropriate genera to these strains.

***Streptococcus* sp. group U-2.** Isolates designated *Streptococcus* sp. group U-2 were anaerobic to aerotolerant, non-

TABLE 4. Comparisons of populations from colonic epithelia and lumen contents of normal and dysenteric pigs

Bacterial genus	% Distribution of bacteria at the indicated location in the following pigs:							
	Normal				Dysenteric			
	Pig 2		Pig 3		Pig 5		Pig 7	
	Wall	Lumen	Wall	Lumen	Wall	Lumen	Wall	Lumen
Anaerovibrio			1.9				13.3	2.3
Acetivibrio					28.8	35.5	10.0	11.3
Bacteroides	20.8	32.5	17.3	11.6	2.2	42.1	16.6	43.1
Bifidobacterium			1.9					2.3
Clostridium					15.5			
Desulfomonas							3.3	
Escherichia		2.5	1.9		40.0			
Eubacterium	1.8	10.0		2.3	2.2	15.5	26.6	13.5
Fusobacterium	1.8	12.5	3.8	16.3			23.8	20.4
Gemminger			1.9					2.2
Lactobacillus			3.8	16.3				9.0
Leptotrichia			1.9					
Peptococcus	1.8							
Peptostreptococcus	1.8							
Selenomonas	1.8		3.8		11.5	6.6	6.6	
Streptococcus	69.8	42.5	59.6	51.1				9.0
No. of isolates (tested)	53	40	52	43	45	45	30	44

TABLE 5. Characteristics of isolates not identified as particular species[a]

Property	Characteristics of the following isolates:					
	Bacteroides sp. group:		*Coprococcus* sp. strain A-1	*Eubacterium* sp. group A-1	*Fusobacterium* sp. group A-1	*Streptococcus* sp. group U-2
	A-1	B-2				
Cellobiose	−	−	w	w	−	a
Esculin pH	w	−	a	+	−	w
Esculin hyd.	+	−	+	+	−	+
Fructose	−	−	a	w	−	a
Galactose	−	w	a	w	−	a
Glucose	a	w	a	w	w	a
Lactose	a	a	−	w	−	−
Maltose	a	a	a	w	−	a
Mannitol	−	−	a	−	−	−
Mannose	a	a	a	−	−	a
Raffinose	a	a	w	−	−	w
Ribose	w	−	−	−	−	−
Salicin	−	a	w	−	−	w
Sorbitol	−	−	w	−	−	−
Starch pH	a	a	−	w	−	−
Starch hyd.	+	+	−	+	−	−
Sucrose	a	a	a	−	−	a
Xylose	w	−	−	−	−	−
Gelatin dig.	+	−	+	−	−	−
Milk	c	−	−	+	−	−
Urease	−	−	−	−	−	±
H_2S	−	−	−	w	−	−
Gas-glucose	−	−	+	−	−	w
Acid-glucose	Saf[b]	Saf	Blf-2	BLf	ba	La(f)
Motility	−	−	−	+	±	−
Bile	a	−	−	+	−	−
Hemolysis	−	−	−	−	−	Weak β
No. of strains tested	17	5	1	14	12	123

[a] Symbols are similar to those used by Holdeman et al. (11): a, acid (pH below 5.5); w, weak acid (pH 5.6 to 6.0). None of the isolates produced acid from arabinose, glycerol, inositol, inulin, or trehalose; none produced indole or catalase or reduced nitrate.

[b] Product abbreviations: (a) acetic, (B,b) butyric, (L,l) lactic, (f) formic, and (S,s) succinic acids and ethanol (2). Capital and lowercase letters indicate major and minor products, respectively. Parentheses indicate reactions of occasional strains in the species.

motile, gram-positive cocci that often occurred in long chains. Cells were often elongated, and the cells of unequal size were found in pairs. All strains were weakly β hemolytic. The hemolytic zone was much more clearly defined from growth on aerobic plates than from growth on plates incubated anaerobically. They had similar physiological characteristics, except for the finding that 116 of the 123 strains (epithelial and luminal isolates) hydrolyzed urea. The ureolytic capability was a stable characteristic. Extracted antigens from 16 of 77 ureolytic strains and 4 of 7 nonureolytic strains (tested) reacted with rabbit antiserum prepared against Lancefield group G streptococci in agarose gel double-diffusion tests. No reactions between antigens from any of the strains tested and the Lancefield group antisera other than for G were detected.

***Bacteroides* spp.** *Bacteriodes* isolates were obligately anaerobic, nonmotile, nonsporulating gram-negative rods. Cells occurred singly, in pairs, and in short chains. The main fermentation product was succinate, but various amounts of acetate and formate were also detected. None of the isolates produced a black pigment on anaerobically incubated blood agar plates. *Bacteroides* sp. group A-1 isolates were similar to *Bacteroides fragilis* but differed from *B. fragilis* because fructose and galactose were not fermented. Hydrogen sulfide and catalase were not detected. *Bacteroides* sp. group B-2 fermented glucose weakly, producing small amounts of succinate, acetate, and formate. Esculin was not hydrolyzed, and growth was inhibited by bile.

***Coprococcus* sp.** The isolate designated *Coprococcus* sp. strain A-1 fermented a variety of carbohyrates, producing mainly *n*-butyrate and smaller amounts of lactate, formate, and ethanol from glucose. Although it resembled *Coprococcus eutactus* (12), it differed from that species by forming very long chains, fermenting mannitol, and failing to hydrolyze starch.

***Eubacterium* sp. group A-1.** These isolates were obligately anaerobic, nonsporing, motile, slightly curved rods with lateral subterminal flagella emanating from the concave surface of the cells. Cells frequently had central or terminal swelling. The cells were gram negative; however, 3-hydroxy fatty acids or 2-keto-3-deoxyoctonic acid, which are characteristic constituents of most gram-negative bacterial lipopolysaccharides, were not detected in the representative strains examined (unpublished data). Major fermentation products in MPYG broth from *Eubacterium* sp. group A-1 cultures were butyrate and lactate. Cultures did not survive heating at 80°C for 10 min.

***Fusobacterium* sp. group A-1.** These isolates were obligately anaerobic, nonsporing, slightly curved rods that were gram negative. Of 12 isolates, 7 were nonmotile. Other differences between motile and nonmotile strains were not noted. All strains were relatively nonreactive in sugar fermentation or biochemical reactions and produced small amounts of acetate during growth in MPYG broth. Examinations for 3-hydroxy fatty acids or for other cellular components that might differentiate gram-negative from gram-

positive cells were not made. Further study of these isolates is in progress.

DISCUSSION

The results of this study support the conclusion that the colons of healthy pigs and pigs with dysentery are populated by a microflora of considerable diversity and that the predominantly gram-positive population that adheres to the epithelium of the proximal colon of healthy weaned pigs is replaced by a predominantly gram-negative population after infection with *T. hyodysenteriae*. The recovery of gram-positive bacteria (71%) as predominant organisms from the colons of normal pigs agrees with results of other studies on swine intestinal and fecal microflora. Russell (21) has reported that 90% of the bacteria isolated from the large intestines of pigs were gram positive and consisted mainly of gram-positive cocci, lactobacilli, eubacteria, and clostridia. Salanitro et al. (22) have reported that over 90% of the fecal microflora of adult swine was gram-positive and consisted of *Eubacterium* sp., *Clostridium* sp., *Propionibacterium acnes*, and facultatively anaerobic streptococci. The streptococci which comprised 44% of the fecal microflora were α hemolytic and were presumptively identified as viridans non-group D streptococci.

Streptococci were the predominant bacteria (54%) isolated from the colonic epithelial tissue of three normal pigs in this study and accounted for 46.9% of the isolates from the colonic luminal contents of two of these pigs. Streptococci were isolated from only two of the four pigs with dysentery and were minor components of the flora. They were 6.2% of the colonic epithelial flora of one pig and 9% of the flora of the colonic luminal contents of a second pig. This may indicate that samples from these two pigs were obtained at a fairly early stage of the disease before changes in the population, including displacement of streptococci by gram-negative organisms (e.g., *A. ethanolgignens*) had occurred.

There are few reports of ureolytic streptococci being obtained from the gastrointestinal tract of swine. Raibaud et al. (17), however, have described 100 ureolytic strains from pigs that belonged to Lancefield group D, with physiological and biochemical characteristics similar to *Streptococcus bovis*. They referred to these urease-positive streptococci as *S. bovis* type C. Of the 123 streptococcus strains we examined with Lancefield group-specific antisera, 20 strains reacted with Lancefield group G antiserum. The rest did not react with any of the Lancefield group antiserum. Representative ureolytic and nonureolytic streptococci strains were examined at the Streptococcus Laboratory of the Centers for Disease Control (Atlanta, Ga.). Their report confirmed the characterization we obtained for these organisms, including the reactions with Lancefield group G antiserum (personal communication, R. R. Facklam).

In our study characterizing the cecal bacteria of normal pigs (19), we reported that of 192 isolates, only 1 isolate was identified as *Streptococcus* sp. A review of the characteristics of this ureolytic isolate indicate that it was similar to *Streptococcus* sp. group U-2 isolated in this study and that it was incorrectly identified as *Streptococcus intermedius*. Several other bacterial species found in high numbers in cecal contents, including *Bacteroides ruminicola* (35.5%) and *Selenomonas ruminantium* (20%), were recovered in low numbers from the colonic epithelium of normal pigs in the present study (4.4% each). Other bacterial species identified in cecal contents, including *Eubacterium aerofaciens*, *Fusobacterium prausnitzii*, *Lactobacillus acidophilus*, *Lactobacillus fermentus*, *Leptotrichia buccalis*, and *Peptostreptococcus productus*, were also recovered from the colons of normal pigs in this study.

TABLE 6. Predominant organisms associated with colonic mucosa of normal and diseased pigs

Bacterial genus	Characteristics		Fermentation products[a]	% flora in the following pigs:	
	Gram stain	Motility		Normal (n = 3)	Diseased (n = 4)
Streptococcus	+	−	La(f)	54.4	6.1
Bacteroides	−	−	Saf	18.4	6.8
Lactobacillus	+	−	L	11.8	0.0
Acetivibrio	−	+	A-2	0.0	27.8
Selenomonas	−	+	Pa	4.4	15.4
Escherichia	−	+	AS	0.7	14.2
Fusobacterium	−	+	BL	2.2	9.9
Eubacterium	+	+	Blf	1.5	8.6

[a] Product abbreviations: (A,a) acetic, (B,b) butyric, (L,l) lactic, (f) formic, (S,s) succinic acids and ethanol (2). Capital and lowercase letters indicate major and minor products, respectively. Parentheses indicate reactions of occasional strains in the species.

Although others (2, 22) have found selenomonads in cecal, colonic, or fecal samples from healthy swine, they were minor components of the microflora. We know of no previous reports indicating that *Selenomonas ruminantium* is a predominant species accompanying a disease process. The fact that *Acetivibrio ethanolgignens* (20) was found consistently in high numbers in the colons of pigs with dysentery, but was not isolated from control pigs, indicates that the physiological conditions in diseased colons are more favorable for growth of this organism. It has not been reported as part of the microflora of any other habitat.

Bacterial species, including *Anaerovibrio lipolytica*, *Bacteroides ruminicola*, *Escherichia coli*, *Leptotrichia buccalis*, *Selenomonas ruminantium*, *Streptococcus* sp., and *Peptostreptococcus productus*, were isolated from the colons of both normal pigs and pigs with dysentery. In contrast, Alexander and Wellstead (2) have reported that none of the bacterial species isolated from normal pigs were recovered from pigs with dysentery after infection with *T. hyodysenteriae*.

Factors influencing these selections in pigs with swine dysentery are not understood, and information concerning the effects of factors such as diet, metabolic products, pH, Eh, or osmolarity on these population changes are meager. Nevertheless, differences in the composition of the colonic flora between healthy and diseased pigs are evident. Major bacterial groups, including streptococci and lactobacilli, associated with the epithelia of normal pigs were aerotolerant, gram positive, and nonmotile (Table 6). Major fermentation products from glucose include acetate, lactate, and formate. Predominant species from pigs with dysentery were obligate anaerobes most of which were gram negative and motile, with the major fermentation products from glucose being acetate, propionate, butyrate, lactate, and ethanol. Isolates of *Acetivibrio ethanolgignens* and *Eubacterium* sp. group A-1 were recovered from pigs with dysentery and not from normal pigs, whereas lactobacilli were recovered only from normal pigs. Our recovery of greater proportions of motile organisms in the samples from dysenteric pigs suggests that motile organisms have a competitive advantage over nonmotile forms for space on the mucosal surface of diseased pigs. It has been shown that compared with wild-type bacteria, nonmotile *Vibrio cholerae* strains have an impaired capacity to penetrate the mucus gel overlying the intestinal epithelium and absorb to underlying intestinal tissue (9).

Inasmuch as many of the organisms that persisted on the mucosal surface of the colon of pigs with dysentery represented an indigenous population, it is difficult to incriminate a specific organism besides *T. hyodysenteriae* in the etiology of swine dysentery. Recent work in our laboratory (S. C. Whipp, J. Pohlenz, D. L. Harris, I. M. Robinson, R. D. Glock, and R. K. Kunkel, Proc. Int. Pig Vet. Soc. 1982, p. 31) has demonstrated that *T. hyodysenteriae* would colonize and express pathogenicity in the colons of gnotobiotic pigs in the absence of other microbial contamination. However, the disease produced in gnotobiotic pigs is not as severe as that which occurs in coventional pigs infected with *T. hyodysenteriae*.

We conclude that the dysenteric state of pigs leads to marked qualitative changes in the populations of bacteria that colonize the colon. Definition of the relationship between these populations and between the host and the microbes is needed for an adequate understanding of the physiological events associated with swine dysentery.

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