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Enteropathogenicity of Various Isolates of *Treponema hyodysenteriae*

JOANN M. KINYON,* D. L. HARRIS, AND R. D. GLOCK

Department of Veterinary Microbiology and Preventive Medicine and Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011*

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Isolates of *Treponema hyodysenteriae* from 25 geographically separated outbreaks of swine dysentery were tested for their ability to produce the disease. Clinical signs and lesions typical of acute swine dysentery were produced in 52 of 68 (75%) susceptible specific pathogen-free pigs that had been orally inoculated with pure cultures of 23 of 25 beta-hemolytic isolates. In addition, 13 weakly beta-hemolytic isolates of nondysentery origin with morphology similar to *T. hyodysenteriae* did not produce disease when orally inoculated into susceptible specific pathogen-free pigs. Two of these latter isolates, Puppy and B296, and one pathogenic, beta-hemolytic isolate failed to produce disease when orally inoculated into puppies.

Swine dysentery (SD) is a mucohemmorrhagic diarrheal disease of swine. The primary lesions of inflammation, excess mucus production, and superficial necrosis of the large intestine result in hemorrhage, dehydration, and death (8). Affected swine herds may experience 90% morbidity and 25% mortality (16). The surviving animals are often unthrifty, due to decreased rate of gain and feed efficiency. The disease has been estimated to cause an annual loss of $34 million to the U.S. pork industry (35).

Although SD was first described in 1921 (45), the etiology of the disease was not demonstrated until 1971 (41, 42). Electron microscopic studies on SD-affected pigs demonstrated the presence of large numbers of spirochetes in the early lesions of the disease (4, 11, 42). Subsequently, this organism was isolated in pure culture and found to induce signs and lesions of SD when orally inoculated into 6- to 8-week-old pigs (10, 18, 41). The organism was propagated on blood agar plates incubated anaerobically and produced zones of beta-hemolysis. The organism was further characterized and named *Treponema hyodysenteriae* (18).

The production of SD by oral inoculation of swine with pure cultures of beta-hemolytic *T. hyodysenteriae* has been reported by many workers (1, 14, 15, 20, 21, 24, 28-30, 34) and is an indication of the involvement of this organism in the etiology of the disease.

Organisms of similar morphology (0.32 to 0.38 μm in diameter; 6 to 8.5 μm long, with seven to nine axial flagella) (19), have been reported in cases of canine diarrhea (5, 13, 23, 31, 40, 44, 46) and in normal swine (41). Isolation of the spirochetes associated with canine diarrhea has not been reported. Taylor and Alexander (41) isolated *T. hyodysenteriae* from normal swine (isolate 4/71) which was not pathogenic for specific pathogen-free (SPF) pigs. This nonpathogen produced less complete hemolysis than the pathogenic isolate A-1. And, in a study involving 334 clinically normal swine from five midwestern U.S. swine herds (SD free), nonpathogenic isolates of *T. hyodysenteriae* were obtained from 20 to 40% of the pigs. The hemolytic pattern of these isolates was like that of isolate 4/71 (J. M. Kinyon, J. Glenn Songer, and D. L. Harris, Congr. Int. Pig Vet. Soc., Ames, Iowa, Abstr. L.8, 1976).

These beta-hemolytic (complete hemolysis) and weakly beta-hemolytic (less complete hemolysis) isolates of *T. hyodysenteriae* differ from the smaller anaerobic treponemes that have been observed by others (17, 19, 36-38) in normal and diseased swine. The latter have been referred to as small spirochetes (17), PR-7 strain (38), PN-5 strain (36), and PF strains (37). They are smaller (4 to 7 μm long and 0.24 to 0.30 μm in diameter, with one to two axial flagella) (17, 19) than *T. hyodysenteriae* and more tightly coiled.

In this study, 25 beta-hemolytic isolates and 13 weakly beta-hemolytic isolates of *T. hyodysenteriae* were orally inoculated into 2-week-old SPF swine to determine the correlation of hemolytic pattern to pathogenicity. In this communication, all isolates will be referred to as

T. hyodysenteriae based on similarity of morphology, as determined by phase-contrast microscopy.

MATERIALS AND METHODS

Isolates. *T. hyodysenteriae* were isolated by previously described methods (19, 39; J. M. Kinyon, M. S. thesis, Iowa State University, Ames, 1974) from intestinal tissue or rectal swabs submitted to this laboratory (Tables 1 and 2). Isolates A-1 and 4/71 were kindly supplied by D. J. Taylor, University of Glasgow, Glasgow, Scotland. Pure cultures of *T. hyodysenteriae* used as inocula had been subpassaged less than 10 times on artificial media.

Isolates were described as weakly beta-hemolytic or beta-hemolytic. The beta-hemolytic reaction is more intense, with a discrete, sharply defined edge, and usually is readily apparent after 2 to 4 days of incubation. The weak beta reaction is less intense, with a poorly defined edge and, frequently, is not apparent until after 6 days of incubation. Both organisms are usually evidenced macroscopically only by the hemolytic zones they produce. The surface growth produced by these organisms is a slight film, without individual distinct colonies.

Media. Blood agar plates used in isolation, propagation, and viable count determinations of *T. hyodysenteriae* were prepared from Trypticase soy agar (TSA) (BBL, Division of Bioquest, Cockeysville, Md.) supplemented with 5% citrated bovine blood. Blood agar plates were prepared the day of use or held anaerobically for 24 h in GasPak jars (BBL, Division of Bioquest, Cockeysville, Md.) before use. A selective isolation medium (39) was used in the latter part of this study. It incorporated 400 m μg of spectinomycin per ml in the TSA with blood (TSA-S400). All agar media were poured to a thickness of 4 to 6 mm (approximately 20 ml of medium per 100-mm petri plate).

Anaerobic atmospheres of approximately 80% H_2 and 20% CO_2 for agar cultures were produced by evacuation and refilling in vented GasPak jars with cold palladium catalyst. All agar cultures were in-

TABLE 1. *Results of oral inoculation of 2-week-old swine with pure cultures of beta-hemolytic Treponema hyodysenteriae isolated from outbreaks of SD*[a]

Isolate no.	Locale[b]	CFU/pig[c]	DPI		Totals	
			CS[d]	PO[e]	SD[f]	Mor[g]
B78	IA	1.8	4	8	2/2	1/2
B137	IA	10.0	7	7	1/2	0/2
B138	IA	0.02	6	7	1/1	0/1
B140	MN	14.0	7	9	7/8	3/8
B153	IN	1.0	9	8	2/2	0/2
B163	MN	1.8	7	7	2/2	1/2
B164	NB	12.0	5	6	2/2	0/2
B169	CAN	0.18	18	18	1/2	0/2
B171	KS	14.0	–	–	0/4	0/4
B173	CAN	1.0	13	13	2/2	2/2
B175	NB	1.8	7	9	2/2	2/2
B204	IA	18.0	8	7	8/9	1/9
B205	CO	1.0	3	4	1/2	0/2
B206	KS	1.4	8	8	1/2	0/2
B211	IL	16.0	8	9	2/2	1/2
B223	CO	1.6	6	7	2/2	1/2
B224	IL	16.0	6	6	2/2	2/2
B228	IA	18.0	6	6	2/2	2/2
B230	IL	140.0	7	6	1/2	1/2
B231	FL	10.0	7	6	3/4	1/4
B234	MO	1.8	8	5	2/4	1/4
B254	NC	1.4	7	7	2/2	1/2
B259	SD	1.8	–	–	0/2	0/2
A-1	GB	100.0	5	4	2/2	1/2
G	MX	100.0	4	3	2/2	2/2

[a] Thirty-five 2-week-old swine inoculated with sterile media were used as uninfected controls.

[b] State (zip code abbreviations) or country from which isolate was obtained. Abbreviations: CAN, Canada; GB, Great Britain; MX, Mexico.

[c] CFU/pig, total number $\times 10^9$ of CFU of *T. hyodysenteriae* received by each pig.

[d] DPI of first observation of clinical signs of SD; –, clinical signs of SD were never observed.

[e] DPI of first phase-microscopic observation of *T. hyodysenteriae* in rectal swab material; –, *T. hyodysenteriae* were never observed.

[f] Numerator, number of pigs that developed clinical signs and lesions of SD; denominator, number of pigs inoculated.

[g] Numerator, number of pigs which became moribund; denominator, number of pigs inoculated.

TABLE 2. *Results of oral inoculation of 2-week-old swine with pure cultures of weakly beta-hemolytic Treponema hyodysenteriae isolated from sources other than SD*[a]

Isolate no.	Locale[b]	Animal[c]	Condition[d]	CFU/pig[e]	SD[f]
B256	IA	S	PWD	10.0	0/4
B296	IA	C	CE	100.0	0/2
B359	MO	S	N	100.0	0/2
B421	MN	S	N	100.0	0/2
B548	IA	S	N	100.0	0/4
B627	WS	S	N	100.0	0/2
B711	IA	S	N	100.0	0/2
B735	IA	S	N	100.0	0/2
B804	MN	S	N	100.0	0/4
B1375	IA	C	N	100.0	0/2
B1555a	IA	S	N	100.0	0/2
Puppy	IA	C	CE	160.0	0/6
4/71	GB	S	N	18.0	0/4

[a] See footnote *a* in Table 1.
[b] See footnote *b* in Table 1.
[c] Species of animal from which isolate was obtained. Abbreviations: S, Swine; C, canine.
[d] Condition of the animal at the time the isolate was obtained. Abbreviations: PWD, Postweaning diarrhea; CE, catarrhal enteritis; N, normal.
[e] See footnote *c* in Table 1.
[f] See footnote *f* in Table 1.

cubated at 42°C for 2 to 4 days (39; J. M. Kinyon, M. S. thesis, 1974).

Trypticase soy broth without dextrose (BBL, Division of Bioquest, Cockeysville, Md.) was prepared by the aerobic method (24) in 180-ml amounts in 500-ml round-bottom flasks. At the time of inoculation, the aerobic atmosphere in the flask was replaced with anaerobic atmosphere by opening the flask under a flow of deoxygenated H_2-CO_2 (50:50). Twenty milliliters of fetal calf serum (GIBCO, Grand Island, N.Y.) and 20 ml of inoculum were added. The flasks were tightly stoppered and incubated at 38°C on a reciprocating shaker (100 rpm) for 36 to 48 h. Purity of cultures was determined by inoculation of thioglycollate broth.

Animals. Swine used in these studies were obtained from two different herds. Yorkshire, Hampshire, and crossbred swine were obtained at 2 weeks of age from the SPF herd maintained at the Veterinary Medical Research Institute at Iowa State University. The SPF Duroc swine were obtained at 2 weeks of age from Sam Kennedy Vegetable and Livestock Co., Clear Lake, Iowa. Both swine herds have been free of SD since their origin, and both were given drug-free feed. The pigs were not vaccinated.

The beagle puppies were obtained at 6 weeks of age from Marshall Research Animal, Inc., Northrose, N.Y. They received modified live distemper and hepatitis vaccine but were not immunized for leptospirosis.

The animals were housed as pairs in previously fumigated isolation cages (4 by 4 by 4 feet [ca. 122 by 122 by 122 cm]) with filtered forced-air systems. Two-week-old pigs were weaned at the beginning of each experiment and were fed an 18% protein starter ration for 2 weeks, followed by a 16% protein grower ration. The beagles were fed Gaines meal (General Foods Corp., White Plains, N.Y.). All feeds were free of added drugs.

Animal inoculation. Pigs and puppies were placed in isolation at least 2 days before the start of an experiment. Feed was withheld for 18 to 24 h before inoculation. Each pair of animals received pure cultures of a single beta-hemolytic or weakly beta-hemolytic isolate propagated in either agar or broth (24). Each pig received 80 to 200 g of mashed blood agar culture or 100 to 200 ml of broth culture on each of 2 consecutive days. Inocula ranged from 10^5 to 10^8 colony-forming units (CFU) per g or ml (Tables 1 and 2). Forty to fifty percent of the culture was given directly (orally via syringe or intragastrically via stomach tube), and the remainder was mixed with a small amount of feed and left for the pigs to eat. Uninfected controls received equal volumes of uninoculated medium on each of 2 consecutive days. Animals were allowed feed ad libitum 24 h after the second inoculation and throughout the experiment. Water was always available.

A total of 71 pairs of pigs were inoculated in 16 separate experiments for this report. Each experiment contained at least one pair of uninfected control animals and one pair of animals inoculated with a beta-hemolytic isolate (known to be pathogenic). Isolates B140, B171, B204, B231, B234, B256, B548, B804, Puppy, and 4/71 were inoculated into more than one pair of pigs in two or more different experiments.

Observations. Animals were observed daily for fecal consistency (normal, soft, loose, or watery); fecal composition (normal, mucus, blood, or mucus and blood); and general condition (normal, depressed, gaunt, or moribund).

The presence or absence of *T. hyodysenteriae*, *Campylobacter* spp., (*Vibrio* spp.), and small spirochetes in the feces was determined once preinocula-

tion, and every 2 days postinoculation (DPI) by examination of rectal swab samples. Rectal swab specimens were collected on sterile, dry, cotton-tipped applicators that were then immersed in 0.5 ml of phosphate-buffered saline, 0.01 M, pH 7.2, and held at 4°C until observed by phase-contrast microscopy (at a magnification of ×630) or cultured or both.

Necropsy. Pigs and puppies were stunned by electrocution and exsanguinated. Postmortem observations of stomach, small intestine, colon, cecum, and rectum, as well as other body organs, were recorded. Portions of the colon and any abnormal body organs were fixed in 10% buffered formalin for observation by light microscopy. These fixed tissues were embedded in paraffin, sectioned (6 μm), and stained with hematoxylin and eosin or Warthin-Starry stains.

A portion of the spiral colon was also placed in a sterile bag for isolation of *T. hyodysenteriae*, isolation of *Salmonella* spp. (32), and for phase-microscopic examination of the mucosa for the presence of *T. hyodysenteriae, Campylobacter* spp., and small spirochetes.

Evaluation. Pigs were considered to be affected with SD when the feces were of a watery consistency and contained blood or mucus or both and high numbers of beta-hemolytic *T. hyodysenteriae*. Additional confirmation of SD was obtained postmortem by the presence of gross and microscopic lesions, as previously described (8, 10, 15, 16, 45).

RESULTS

Enteropathogenicity—beta-hemolytic isolates. SD was produced in 52 of 68 pigs, which were orally inoculated with pure cultures of beta-hemolytic isolates of *T. hyodysenteriae* (Table 1). Twenty-three of the twenty-five beta-hemolytic isolates produced SD in at least one of two inoculated pigs. Isolates B140, B204, B231, and B234 produced SD in seven of eight, eight of nine, three of four, and two of four inoculated pigs, respectively. None of the four pigs that had been inoculated with beta-hemolytic isolate B171 and neither of the two pigs inoculated with beta-hemolytic isolate B259 developed SD.

In addition to the results in Table 1, two beta-hemolytic isolates of *T. hyodysenteriae* were subcultured in vitro more than 10 times before oral inoculation of pigs. Isolate B204, after 25 in vitro passages, produced typical signs and lesions of SD in four of four inoculated pigs. Strain B78 (the type species), after 35 in vitro passages, did not produce disease in two pigs. The beta-hemolytic pattern of B204 and B78 was not altered with increased number of in vitro passages.

Clinical signs of SD and *T. hyodysenteriae* in rectal swab material were first observed in affected pigs at an average of 7 DPI (range, 3 to 18 DPI) (Table 1). The early signs of SD usually included depression and watery diarrhea, followed within 18 to 24 h by the acute signs of gauntness and diarrhea with mucus and blood. Of the 52 pigs that developed SD after oral inoculation with pure cultures, 23 (44%) became moribund. The first pig of an inoculated pair to reach the acute or moribund stage of the disease was killed for necropsy examination. When possible, the second pig of each inoculated pair was observed for the 30-day period. In several instances, the second pig of the pair also developed severe SD and was killed and necropsied when moribund. In several other cases the second pigs developed clinical signs of SD, recovered, and remained normal throughout the remainder of the 30-day observation period. Still others developed severe SD, recovered, and then exacerbated (with clinical signs of SD and large numbers of *T. hyodysenteriae* in the feces) two to four times in 4- to 10-day cycles.

Lesions typical of SD were noted in all pigs that had clinical signs of the disease. These lesions were limited to the large intestine. The serosal surface of the colon was frequently hyperemic and, occasionally, pale nodules were present. The mesentery and colonic tissues were often edematous. Gross lesions of the mucosal surface of the colon varied from mucosal hyperemia and catarrhal inflammation to pseudomembrane accumulation and necrosis. The cecum was occasionally involved with gross lesions similar to those seen in the colon. Microscopically, the colonic crypts appeared dilated, and goblet cells were increased in number (Fig. 1). The mucosal surface sometimes completely lacked epithelial covering. In such cases the surface was covered with an adherent layer of mucus, fibrin, erythrocytes and leukocytes, and bacteria (Fig. 2). The vessels of the lamina propria and submucosa appeared congested and, frequently, areas of hemorrhage were found. Leukocytic infiltration of the tissues was noted in varying degrees, as was pavementing of leukocytes in the vessels of the mucosa and submucosa.

No other gross lesions were observed in these pigs, except in an isolate A-1-inoculated pig, in which lesions suggestive of mycoplasma pneumonia were present in the cardiac lobes of the lung, and an isolate B234-inoculated pig, which had splenomegaly and ascites. The two pigs that were inoculated with isolate B173 developed lesions of SD in the colon and cecum, and large numbers of *T. hyodysenteriae* were present in the mucosa. However, the small intestine of each pig was distended with bile-colored watery fluid and gas. *Escherichia coli*

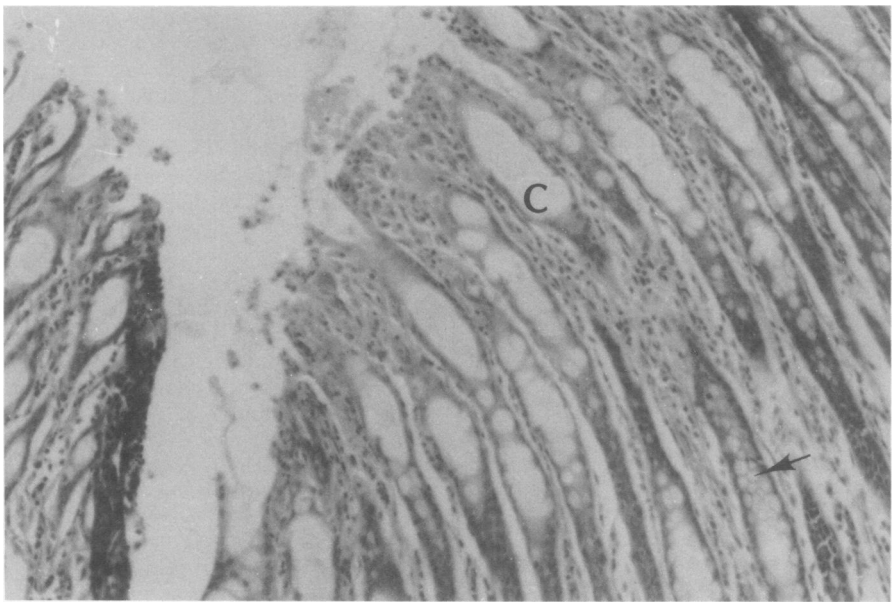

FIG. 1. *Colonic mucosa (of a pig infected with beta-hemolytic T. hyodysenteriae), with dilated crypts (c), goblet cell hyperplasia (arrow), and erosion of the epithelium. Hematoxylin and eosin stain. (×360)*

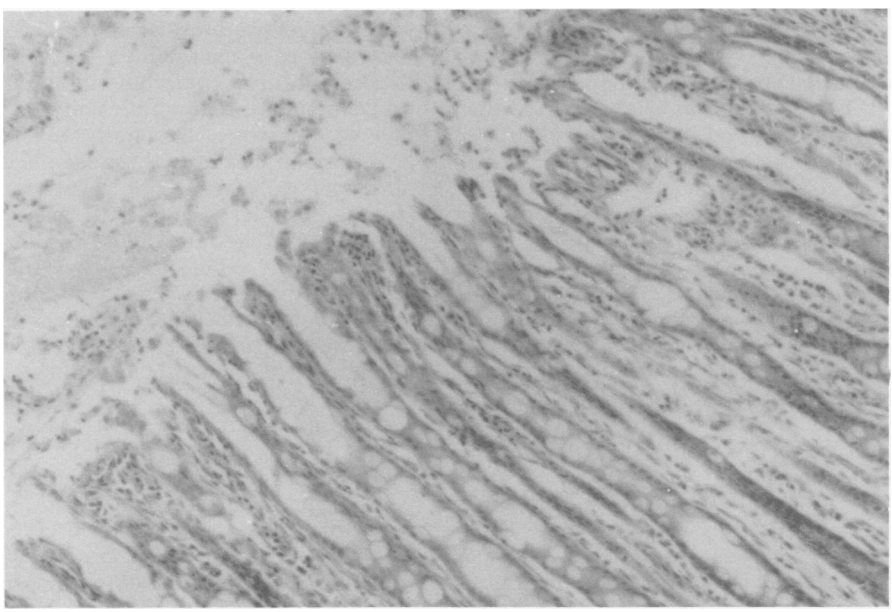

FIG. 2. *Colonic mucosa and lumen, which contains mucus, fibrin, sloughed epithelial cells, and leukocytes. Hematoxylin and eosin stain. (×145)*

was present in fluids from the jejunum at 10^7 CFU/ml.

T. hyodysenteriae (2 to 30 per field) were observed by phase microscopy in scrapings from the colonic mucosa of all necropsied pigs affected with SD (Fig. 3). The organisms frequently appeared to be attached to, and possibly penetrating, epithelial cells. Beta-hemolytic *T. hyodysenteriae* were reisolated from all affected pigs. The average number of *T. hy-*

odysenteriae in the colonic mucosa was 8×10^7 CFU/g.

Isolate B204 (beta-hemolytic) was orally inoculated into two 6-week-old beagle puppies. The puppies remained normal throughout a 30-day observation period, and *T. hyodysenteriae* were not observed in their feces.

Enteropathogenicity—weakly beta-hemolytic isolates. Cultures of 13 weakly beta-hemolytic *T. hyodysenteriae* isolates were orally inoculated into 38 pigs. In addition, the Puppy isolate and isolate B296 were orally inoculated into eight puppies. None of these pigs or puppies developed signs or lesions of enteric disease (Table 2). Pigs (two of four) that were inoculated with isolate 4/71 experienced transient watery diarrhea.

Controls. Thirty-five uninfected control pigs were used in these studies. Some pigs infrequently developed transitory signs of postweaning colibacillosis just after introduction to the isolation cage. This diarrhea was usually of short duration (1 to 2 days). *T. hyodysenteriae* were seen in high numbers in two control pigs that had been affected with postweaning diarrhea for 4 to 7 days. One of two attempts to isolate these organisms was successful (weakly beta-hemolytic isolate B256). Macroscopic lesions included gas and watery ingesta in the small intestine and colon. Fluids from the jejunum contained 10^6 to 10^7 CFU of *E. coli* per ml. All other control swine remained clinically normal, and *T. hyodysenteriae* were not observed in feces or in the colonic mucosa.

This study has been conducted over a 4-year period. For the first 3 years, the sensitivity of culture for *T. hyodysenteriae* and phase-microscopic observation of *T. hyodysenteriae* were approximately equal. During that time, few control or preinoculation pigs were observed to harbor *T. hyodysenteriae*. However, in the last year, with the advantages of TSA-S400, weakly beta-hemolytic *T. hyodysenteriae* have been isolated on at least one occasion from 80 to 100% of the control and preinoculation pigs used in the study. The organisms are present in the feces or colonic mucosa of swine in low numbers (10^2 or 10^3 CFU/g), and primary isolation frequently requires longer (6 to 8 days) incubation.

The four uninfected beagle controls also remained clinically normal and they occasionally shed low numbers of *T. hyodysenteriae*. These organisms were isolated (weakly beta-hemolytic isolate B1375) from rectal swab material using TSA-S400.

Other microflora. *Campylobacter* spp. and small spirochetes were observed in low numbers (one to three per field) in rectal swab

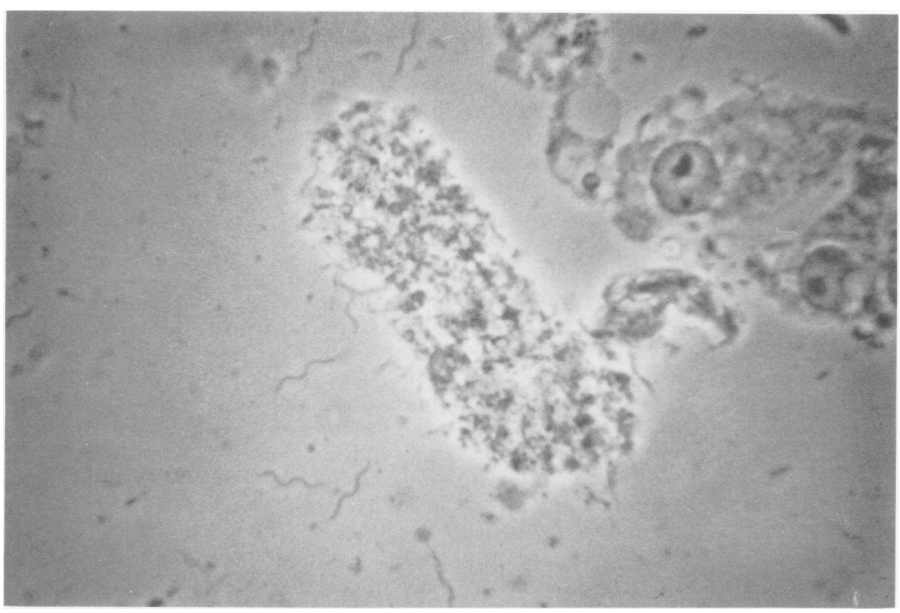

FIG. 3. *Phase-contrast photomicrograph of wet-mount direct smear from colonic mucosa of a pig infected with beta-hemolytic T. hyodysenteriae. (×1,400)*

samples of all pigs in this study, although not always from each rectal swab sample. There were high numbers of *Campylobacter* spp. (8 to 10 per field) present in inoculated pigs when large numbers of *T. hyodysenteriae* were also present. These were frequently isolated but were not identified to species.

Salmonella spp. were not isolated from any pigs in this study. Two of the uninfected control beagles harbored *Salmonella schwarzengrund*.

Balantidium coli was observed from rectal swab material in one pig inoculated with beta-hemolytic isolate B254 and in one pig inoculated with beta-hemolytic isolate B205.

DISCUSSION

Beta-hemolytic isolates. The transmission experiments in these studies indicate the significant role of beta-hemolytic isolates of *T. hyodysenteriae* in the etiology of SD. Twenty-three of twenty-five beta-hemolytic isolates produced SD when orally inoculated into susceptible 2-week-old swine from two Iowa SPF herds. The isolates were from geographically separated outbreaks of SD (Colorado, Florida, Indiana, Illinois, Iowa, Kansas, Minnesota, Nebraska, North Carolina, Canada, Great Britain, and Mexico).

Pure cultures of *T. hyodysenteriae* B78 had been previously used to produce SD in 6- to 8-week-old pigs (10, 18). In this study, the 2-week-old pig model was an economical and practical method for the determination of pathogenicity of the isolates. This model is perhaps not ideal for study of a disease that occurs primarily in 7- to 14-week-old pigs. However, SD does occur naturally in the suckling piglet (2, 16, 26).

Clinically and pathologically, the disease produced was like the natural infection in weanling aged pigs (2, 16, 26, 45). The incubation period for production of SD by pure cultures of *T. hyodysenteriae* in the present studies was similar to the incubation period reported for (i) the natural infection (16, 26, 45) (ii) crude SD infection (6, 11, 16, 33), and (iii) pure-culture infection (10, 14, 17, 21, 41). Watery grey diarrhea was usually observed for less than 1 day before mucus and blood were seen. The pigs then became rapidly dehydrated and gaunt. Several pigs that had developed SD recovered and remained normal, some continued to show signs of SD and *T. hyodysenteriae* in the feces over a 30-day observation period, and others became moribund. These facts confirm the production of SD in all its ramifications by oral inoculation of SPF pigs with pure cultures of *T. hyodysenteriae*. Reports concerning the oral inoculation of susceptible pigs with pure cultures of *Campylobacter* spp. (*V. coli*) (8) have never satisfactorily demonstrated its role in the etiology of the disease (6–8, 10, 14, 22, 43).

The role of stress in the development of SD has been recognized (2, 8, 45). Eriksen and Anderson (9) reported the production of SD only in swine that were receiving daily intramuscular injections of corticosteroids. The pigs used in this study were naturally stressed by the 24-h starvation period before inoculation, the experimental environment, and the handling involved in inoculation and in collection of rectal swabs. Typical signs and lesions of SD were produced even though corticosteroids were not given.

SD has been observed superimposed on other enteric infections such as salmonellosis (12) and trichuriasis (3). However, those pathogens were not isolated or observed in any of the pigs in this study. Two pigs inoculated with beta-hemolytic isolate B173 developed SD while experiencing post-weaning colibacillosis.

Hudson et al. (20) have shown attenuation of pathogenic isolate A-1 (beta-hemolytic) after 80 laboratory passages. The results of this study also indicate loss of enteropathogenicity after repeated laboratory passage.

Weakly beta-hemolytic isolates. None of the 13 weakly beta-hemolytic isolates used in these studies produced enteric disease in 2-week-old SPF swine. This is evidence that the hemolytic pattern of isolates of *T. hyodysenteriae* with few laboratory passages may be an indicator of the enteropathogenicity of that isolate. The correlation is not direct because beta-hemolytic isolates lose pathogenicity but retain beta-hemolysis after repeated laboratory passage. The role, if any, of the hemolysin in the mechanism of infection has not been determined.

The isolation of nonpathogenic weakly beta-hemolytic *T. hyodysenteriae* (isolate B256) from a pig with postweaning colibacillosis is somewhat contradictory to a report by Akkermans and Pomper (1). They studied 169 cases of diarrhea in swine with diseases other than SD, and did not detect *T. hyodysenteriae* using a fluorescent-antibody test (FAT). The isolation of B256 does agree with an observation of Leach et al. (25). They observed increased numbers of spirochetes in magnesium sulfate-stimulated diarrhea of rats and dogs. Leach et al. postulated that this phenomenon was a result of dislodgement of the organisms from the crypts of the mucosa as fluids were being lost.

Mechow (27) used the FAT in a study of swine operations in Germany. He demonstrated fluorescing organisms with the morphology of *T. hyodysenteriae* in herds that were diagnosed as not having clinical SD. He concluded that those pigs were carriers of pathogenic *T. hyodysenteriae* and responsible for subclinical maintenance of the disease within the herd. However, the specificity of the FAT was not stated, and it is possible that the fluorescing organisms were nonpathogenic types.

This is the first report of the isolation of spirochetes (Puppy isolate and B296) from enteritis in dogs. Previous workers (5, 13, 23, 31, 44, 46) have noted organisms with the morphology of *T. hyodysenteriae* in the stools of diarrheic dogs but have not successfully cultured them. The reports of van Ulsen and Lambers (44) and Goudswaard and Cornelisse (13), in which spirochetes with the morphology of *T. hyodysenteriae* were observed by a FAT of diarrheic stools of affected dogs, are not surprising, as cross-reactivity of rabbit antisera to beta-hemolytic isolates of *T. hyodysenteriae* and antigen prepared from the Puppy isolate has been observed in a FAT (R. D. Glock, Proc. World Vet. Congr., Thessaloniki, Greece, 1975). The exact role of nonpathogenic (weakly beta-hemolytic) *T. hyodysenteriae* in canine diarrhea has not been determined, but these organisms were observed and have been isolated from uninfected clinically normal beagles (isolate B1375).

Diagnosis of SD must rely, in part, upon the establishment of the presence of pathogenic types of *T. hyodysenteriae*. Direct microscopic examination of material from the suspect case will not fulfill this requirement because pathogenic and nonpathogenic types are morphologically identical. Neither will the FAT serve to adequately establish the presence of pathogenic types, because pathogenic and nonpathogenic types cross-react. Culture appears to be the most satisfactory method because it is possible to separate pathogenic from nonpathogenic types by hemolytic pattern.

It is possible that nonpathogenic *T. hyodysenteriae* are part of the normal flora in both swine and dogs. Studies are in progress to determine whether significant metabolic differences exist between pathogenic and nonpathogenic types that might suggest a new species designation for the nonpathogens.

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