Comparison of Stained Smears and Culturing for Identification of *Treponema hyodysenteriae*†

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A comparative study was made of stained fecal smears and cultured fecal swabs for identification of the large spirochetes *Treponema hyodysenteriae* and *Treponema innocens*. Feces were obtained by swabbing rectums, colons, and stools of nonexposed swine and swine experimentally exposed to swine dysentery. In this study there was a significant ($P < 0.001$) correlation between the observation of one or more large spirochetes on stained slides and obtaining either a strong or a weak beta-hemolytic reaction in culture. A significant ($P < 0.001$) correlation was also found between the observation of one or more large spirochetes on stained smears or obtaining either a strong or a weak beta-hemolytic reaction in culture and the occurrence of either nonhemorrhagic or hemorrhagic diarrhea in the swine. In the diarrheic swine at the time of swabbing, 157 of 393 samples (40%) were negative for both the presence of large spirochetes on stained smears and the production of either a strong or a weak beta-hemolytic reaction; in nondiarrheic swine, 42 of 278 samples (15.1%) were positive in stained smears and 32 of 268 samples (11.9%) were positive by culturing. In swine infected with swine dysentery, 17 of 1,011 samples produced weak beta-hemolytic reactions, and in swine infected with nonpathogenic large spirochetes of *T. innocens*, three of 34 samples produced strong beta-hemolytic reactions. It was concluded from this study that neither staining rectal smears nor culturing rectal swabs is sufficient, either together or alone, for the diagnosis of swine dysentery; however, these laboratory methods could be highly supportive of a diagnosis of swine dysentery in swine with clinical signs and lesions of the disease.

Much progress has been made in the in vitro propagation of the etiological agent of swine dysentery, *Treponema hyodysenteriae* (2, 11). A major breakthrough in the culturing of spirochetes from the intestinal tracts of swine was made with the addition of spectinomycin, an excellent inhibitor of microbial flora, to blood agar (10). Isolates of large spirochetes producing a strong beta-hemolytic reaction when grown anaerobically on blood agar have been associated with *T. hyodysenteriae* (7), whereas isolates of large spirochetes producing a weak beta-hemolytic reaction on blood agar have been associated with a nonpathogenic spirochete harbored in swine, *Treponema innocens* (6). Some investigators have claimed that a confirmative diagnosis of dysentery in swine with clinical signs and lesions or in asymptomatic carriers can be achieved only by the cultural isolation of *T. hyodysenteriae* (3). The necessity for this requirement has been questioned, since it de-

pends heavily upon the subjective interpretation of hemolysis. In addition, some veterinary diagnosticians do not have the facilities or assistance for culturing. Furthermore, it has been reported that isolates of large spirochetes from swine suspected of being pathogenic produce a weak beta-hemolytic reaction when cultured (J. J. Andrews and L. J. Hoffman, Abstr. Proc. North Central Vet. Diagn. Meet. 1982, p. 20), and isolates suspected of being nonpathogenic produce a strong beta-hemolytic reaction (4; R. J. Lysons, R. M. Lemeke, M. R. Burrows, and J. Bew, Proc. Int. Symp. Vet. Lab. Diag. 2nd, 1980, p. 118).

The purpose of this study was to determine from rectal, colonic, and stool swabs of swine exposed to swine dysentery (i) the relationship between the observation of large spirochetes resembling *T. hyodysenteriae* on stained smears and the isolation of *T. hyodysenteriae* by cultural methods and (ii) the relationship of these parameters to the condition of the stool at the time of swabbing, the site of swabbing, and the stage in the clinical course of the disease. These

parameters have not been compared before, although culturing for *T. hyodysenteriae* has been compared with the serological response to this organism (1, 5). A third objective introduced after encountering a nonpathogenic large spirochete, *T. innocens*, in swine with soft stools was to differentiate these from a pathogenic spirochete, i.e., *T. hyodysenteriae*.

MATERIALS AND METHODS

Source of swine for smears and swabs. A total of 1,045 swabs for staining smears and culturing were collected from 170 feeder swine exposed to swine dysentery (9) and 15 nonexposed swine; 944 rectal swabs were collected from live swine, 25 rectal and 48 colonic swabs were collected from dead swine, and 28 stool swabs were collected from live swine. These swine were involved in three experiments for the evaluation of several therapeutic compounds for swine dysentery. The source of the swine harboring the nonpathogenic spirochete *T. innocens* were the nonexposed, nonmedicated swine in the second experiment.

Swabbing site and schedule. Swine were usually swabbed weekly, regardless of whether they had diarrhea. If swine developed diarrhea between schedules, they were swabbed rectally or their stools on the floor were swabbed. Some swine were swabbed during treatment. The emphasis after withdrawal of medication was on the detection of carriers. A scraping of feces from the mid-colonic mucosa, in addition to a rectal swab, was obtained from 25 swine that died in the second experiment. In the third experiment, swine were swabbed rectally every day during the incubation period.

Staining smears and culturing swabs. Smears made from the swabs were stained with Victoria blue 4-R stain (8) and observed microscopically under oil immersion (×1,000) to identify large spirochetes; the number of spirochetes per 10 oil immersion fields (OIFs) was counted and averaged. The same swabs were streaked at least five times over a section of a
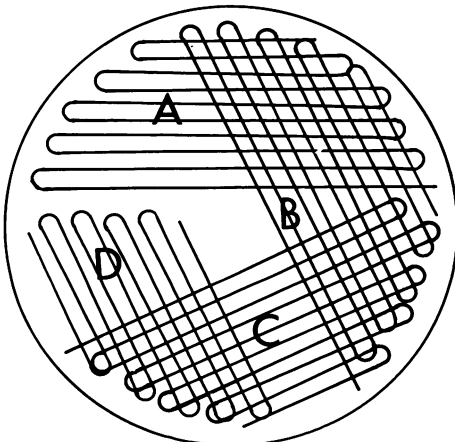

FIG. 1. Pattern for initial streaking (A) and subsequent restreaking (B, C, and D) sections of blood agar plates with rectal, colonic, and stool swabs.
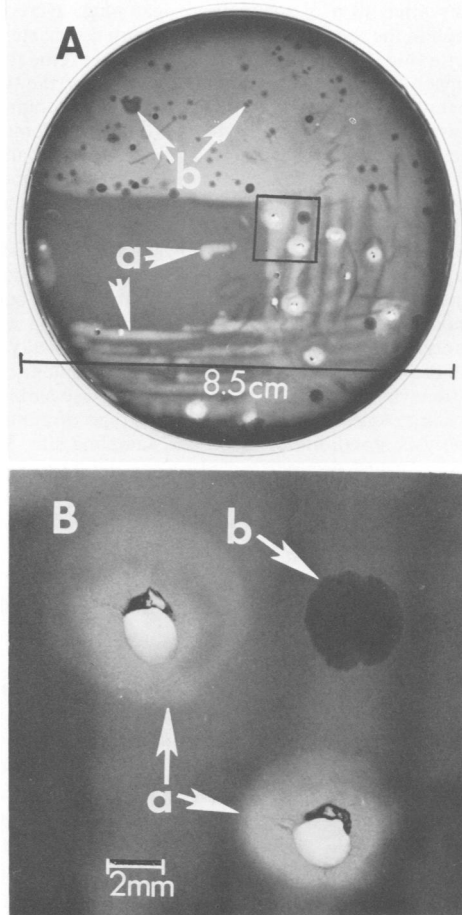

FIG. 2. (A) Photograph of a blood agar plate streaked with a rectal swab from a pig infected with the pathogenic large spirochete *T. hyodysenteriae* and having clinical signs of swine dysentery. (a) Strong beta-hemolytic reaction; (b) fecal microbial contaminant. (B) Magnification of rectangle in (A) showing strong beta-hemolytic reaction surrounding area from which agar plugs were removed. Notice the intensity and width of strong beta-hemolysis around the holes and the discrete edge where the hemolysis ceased (a). (b) Fecal microbial contaminant.

Trypticase soy agar (BBL Microbiology Systems) plate containing 5% sheep blood and 400 µg of spectinomycin per ml (Fig. 1, section A). The plates were restreaked three more times. The inoculum for each additional restreaking (Fig. 1, sections B through D) was obtained by passing a sterilized inoculating loop back through the previously streaked section at least five times. The plates were incubated anaerobically at 42°C in an atmosphere of 80% H_2–20% CO_2 as previously described (10). The interpretation of the growth and type of hemolysis was made independently by one of us (W. H. F.) who was intentionally unfamiliar with the clinical condition of the swine at the time of swabbing. If areas of beta-hemolysis developed on the

plates after 48 h of incubation, agar plugs were removed in the areas of the hemolysis with a 1-ml sterile pipette (Fig. 2A and 3A) and the plates were reincubated anaerobically and reexamined 24 h later for the type of beta-hemolysis around the holes (personal communication, J. M. Kinyon, Iowa State University, Ames). A strong beta-hemolytic reaction was characterized by a brightness around the hole, with a more discrete and defined edge where it ended (Fig. 2A and B), whereas a weak beta-hemolytic reaction was characterized by a lower intensity of brightness around the hole, with a poorly defined boundary where it ended (Fig. 3A and B). Spirochetal growth in the area of beta-hemolysis was confirmed by staining a scraping with Victoria blue 4-R and observing it microscopically.

Statistical analysis. A Pearson product-moment correlation was calculated between each of these parameters: large spirochete count per OIF, type of cultural hemolysis, condition of stool, and sampling site. The chi-square test was performed on the differences between stained smears and culturing within and between the rectum or colon.

RESULTS

There was a highly significant ($P < 0.001$) correlation ($r = 0.48$) between the observation of one or more large spirochetes resembling *T. hyodysenteriae* per OIF in smears stained with Victoria blue 4-R and obtaining either a strong or a weak beta-hemolytic reaction characteristic of spirochetal growth on blood agar. However, there were 67 samples which had one or more large spirochetes per OIF in stained smears but did not grow in vitro (Table 1) and 57 samples which produced either strong or weak beta-hemolytic reactions but had no large spirochetes in stained smears (Table 2). Of the 1,045 samples collected, 278 (26.6%) had one or more large spirochetes per OIF (Table 1), and 268 (25.6%) produced either strong or weak beta-hemolytic reactions in culture (Table 2). Of the 268 samples that produced either strong or weak beta-hemolytic reactions on blood agar, a total of 246
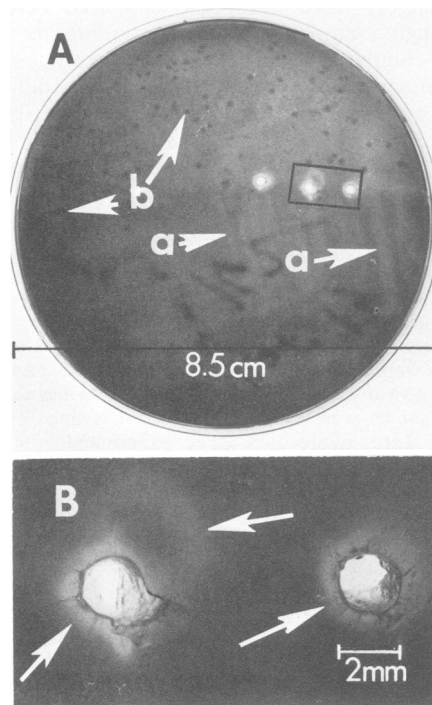
FIG. 3. (A) Photograph of a blood agar plate streaked with a rectal swab from a pig harboring the nonpathogenic large spirochete *T. innocens* and having a soft stool. Notice the weak beta-hemolytic reaction (a) compared with that shown in Fig. 2A. (b) Fecal microbial contaminant. (B) Magnification of rectangle in (A) showing weak beta-hemolytic reaction surrounding area from which agar plugs were removed. Notice the less intense and narrower band of hemolysis around the holes and the less definite edge where the hemolysis ended (arrows) compared with that shown in Fig. 2B.

produced strong beta-hemolytic reactions. With each increase in large spirochete count per OIF,

TABLE 1. Relationship of cultural hemolytic type and type of diarrhea with large spirochete count per OIF in fecal smears from swine exposed to swine dysentery

Large spirochete count per OIF[a]	No. (%) of samples	No. (%) of samples showing[b]:			No. (%) of samples from pigs with following type of diarrhea:		
		No growth	Strong beta	Weak beta	None	Nonhemor-rhagic	Hemorrhagic
1–2	100 (10)	48 (48)	46 (46)	6 (6)	30 (30)	47 (47)	23 (23)
3–9	110 (11)	18 (16)	92 (84)	0 (0)	11 (10)	42 (38)	57 (52)
>10	68 (6)	1 (2)	62 (91)	5 (7)	1 (2)	24 (35)	43 (63)
Subtotal (%)	278 (27)	67 (24)	200 (72)	11 (4)	42 (15)	113 (41)	123 (44)
0	767 (73)	710 (93)	46 (6)	11 (1)	610 (80)	117 (15)	40 (5)
Total (%)	1,045	777 (74)	246 (24)	22 (2)	652 (62)	230 (22)	163 (16)

[a] 1–2, Few; 3–9, moderate; >10, many.
[b] beta, Beta-hemolysis.

TABLE 2. Relationship of large spirochete count per OIF in feces and type of diarrhea to type of cultural hemolysis from feces of swine exposed to swine dysentery

Type of cultural hemolysis[a]	No. (%) of samples	No. (%) of samples with following large spirochete count per OIF[b]:				No. (%) of samples from pigs with following type of diarrhea:		
		0	1–2	3–9	>10	None	Nonhemorrhagic	Hemorrhagic
Strong beta	246 (24)[b]	46 (19)	46 (19)	92 (37)	62 (25)	26 (10)	100 (41)	120 (49)
Weak beta	22 (2)	11 (50)	6 (27)	0 (0)	5 (23)	6 (28)	8 (36)	8 (36)
Subtotal	268 (26)	57 (21)	52 (20)	92 (34)	67 (25)	32 (12)	108 (40)	128 (48)
No growth	777 (74)	710 (91)	48 (6)	18 (2)	1 (1)	620 (80)	122 (16)	35 (4)
Total	1,045	767 (73)	100 (20)	110 (11)	68 (7)	652 (62)	230 (22)	163 (16)

[a] beta, Beta-hemolysis.
[b] 1–2, Few; 3–9, moderate; >10, many.

there was a concurrent increase in the percentage of samples producing strong beta-hemolytic reactions and a decrease in the percentage with no growth on culture (Table 1).

There was a highly significant ($p < 0.001$) correlation ($r = 0.48$) between the presence of either nonhemorrhagic or hemorrhagic diarrhea at the time of swabbing and the presence of one or more large spirochetes per OIF in stained smears. There was also a highly significant ($P < 0.001$) and greater correlation ($r = 0.59$) between either nonhemorrhagic or hemorrhagic diarrhea and obtaining either a strong or a weak beta-hemolytic reaction upon culturing. More of the stained smears with only a few large spirochetes per OIF were from swine with either nonhemorrhagic diarrhea or no diarrhea at the time of swabbing, whereas many of the stained smears with a moderate number or many large spirochetes per OIF were from swine with hemorrhagic diarrhea (Tables 1 and 3).

Of the 246 samples that produced strong beta-hemolytic reactions, 220 (89%) were from swine with either nonhemorrhagic or hemorrhagic diarrhea at the time of swabbing (Table 3). How-

ever, 42 of the 278 stained smears (15.1%) that had one or more large spirochetes per OIF (Table 1) and 32 of the 268 swabs (11.9%) that produced either a strong or a weak beta-hemolytic reaction (Table 2) were from swine with no diarrhea at the time of swabbing. Likewise, 157 of the 393 swabs (40%) from swine with either nonhemorrhagic or hemorrhagic diarrhea at the time of swabbing were negative for large spirochetes on stained smears, and an equal number of samples were negative for cultural growth (Table 3).

There was no difference between swabs of colonic mucosa and rectal swabs from 25 swine that died of swine dysentery in the observation of large spirochetes on stained smears or obtaining cultural growth (Table 4). The differences between the rectum and colon in sampling with each of the methods and the differences between the two methods in sampling the same organ were not significant by the chi-square test ($P > 0.05$). Of the 28 swabs of stools recently defecated by diarrheic swine, 14 had one or more large spirochetes per OIF, whereas only 9 were positive for culturing and produced strong beta-

TABLE 3. Relationship of large spirochete count per OIF in feces and type of cultural hemolysis to type of diarrhea in swine exposed to swine dysentery

Type of diarrhea	No. (%) of samples	No. (%) of samples with following large spirochete count per OIF[a]:				Cultural growth as no. (%) of samples with following type of hemolysis:		
		0	1–2	3–9	>10	No growth	Strong beta	Weak beta
Nonhemorrhagic	230 (22)	117 (51)	47 (20)	42 (18)	24 (11)	122 (53)	100 (44)	8 (3)
Hemorrhagic	163 (16)	40 (25)	23 (14)	47 (35)	43 (26)	35 (21)	120 (74)	8 (5)
Subtotal	393 (38)	157 (40)	70 (18)	99 (25)	67 (17)	157 (40)	220 (56)	16 (4)
None	652 (62)	610 (93)	30 (4)	11 (2)	1 (1)	620 (95)	26 (4)	6 (1)
Total	1,045	767 (73)	100 (10)	110 (11)	68 (6)	777 (74)	246 (24)	22 (2)

[a] 1–2, Few; 3–9, moderate; >10, many.

TABLE 4. Results of observing stained smears under oil immersion for large spirochetes and culturing for *T. hyodysenteriae* from rectums and colons of 25 swine that died of swine dysentery

Combinations of results	No. of swine with:	
	Large spirochetes in stained smears	Cultural growth
Rectum⁺, Colon⁺	15	12
Rectum⁻, Colon⁺	7	6
Rectum⁺, Colon⁻	1	3
Rectum⁻, Colon⁻	2	4

hemolytic reactions.

Most of the samples with large spirochetes on stained smears or producing strong beta-hemolytic reactions were obtained late in the incubation period or during the drug withdrawal period, as was true for most of the samples from swine with normal stools. All of the swabs collected during the incubation period which were positive on stained smears and cultural growths were collected within 1 day of the onset of diarrhea. There were few positive stained smears and positive cultural growths during treatment.

The highest number of large spirochetes seen per OIF in stained smears was observed at the onset of diarrhea after the incubation period (Fig. 4). Numbers this high were not seen subsequently in the course of the disease or after the withdrawal of drugs. Shriveled and uncurled large spirochetes, suspected of being dead, were seen late in the clinical course of swine dysentery and frequently in the rectal swabs of swine with 1- or 2-day recurrences of swine dysentery.

Nonpathogenic large spirochetes resembling *T. innocens* were isolated from the nonexposed, nonmedicated control swine in the second experiment which had soft stools at the time of swabbing. Defecation of soft stools in these nonexposed swine occurred 2 weeks after the initial exposure of the exposed swine in the experiment to infective swine dysentery inoculum. These swine did not develop swine dysentery, and the soft stool receded within 1 week. Although five of the eight swabs from these swine produced weak beta-hemolytic reactions, three of the samples produced strong beta-hemolytic reactions; however, samples producing weak beta-hemolytic reactions were not isolated from any of the other swine exposed to swine dysentery in the experiment, nor were combinations of strong and weak beta-hemolytic reactions seen on the same blood agar plate from these swine.

DISCUSSION

Although most of the cultured samples of *T. hyodysenteriae* in this study produced strong

beta-hemolytic reactions and a majority of the cultured samples of *T. innocens* produced weak beta-hemolytic reactions, the only definite criterion for differentiating nonpathogenic spirochetosis from swine dysentery was that the nonexposed nonmedicated swine harboring the nonpathogenic large spirochete *T. innocens* did not subsequently develop severe diarrhea with mucus and blood, although the soft stools in these swine resembled the type of stool frequently observed at the onset of swine dysentery.

The removal of an agar plug in an area of confluent spirochetal growth increased the amount of hemolysis surrounding the hole, thus making it easier to distinguish the two types of beta-hemolysis. It was thought that this occurred because of the increased surface area of the agar to the gases in the anaerobic jar.

The occasional cultural development of weak beta-hemolysis from rectal swabs of swine with dysentery may have resulted from too few *T. hyodysenteriae* organisms in the inoculum, since many, but not all, of the corresponding stained slides had few or no large spirochetes per OIF. The corresponding stained slides from swabs which occasionally produced weak beta-hemolytic reactions from swine with hemorrhagic stools also had few spirochetes per OIF. The opposite did not appear to be true with the occasional development of a strong beta-hemolytic reaction with nonpathogenic spirochetes, *T. innocens*. Greater success in isolating *T. hyodysenteriae* from rectal swabs of swine with diarrhea than from their diarrheic feces on the floor may have been because the spirochetes
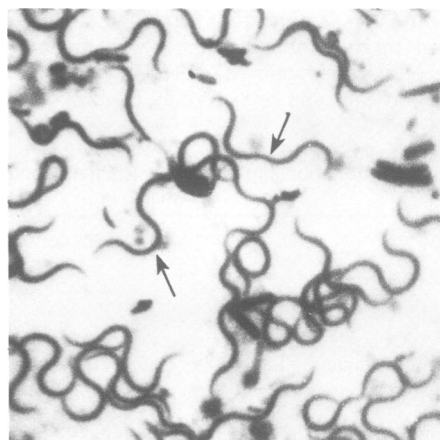

FIG. 4. Stained (Victoria blue 4-R) smear of rectal swab from a pig infected with swine dysentery at the onset of diarrhea. Notice the numerous large spirochetes, *Treponema hyodysenteriae* (arrows). Magnification, ×1,620.

had a tendency to collect in the folds of the rectum, whereas they were diluted in the feces. The spirochetes should not have died in the feces, since the samples were collected shortly after defecation.

There probably has to be a minimum number of large spirochetes in the inoculum for the in vitro growth of either *T. hyodysenteriae* or *T. innocens*. In contrast to restreaking other microorganisms, the inoculating loop in restreaking spirochetes had to be passed back through the section previously streaked at least five times in each restreaking to bring forward an adequate number of spirochetes for growth in the section of final restreaking. Concurrently, the fecal microbial flora was also diluted sufficiently to be inhibited by the spectinomycin in this section.

Making a diagnosis of swine dysentery by the observation of clinical signs, lesions, and large spirochetes in stained smears is relatively easy. Under these conditions, cultural growth with strong beta-hemolysis is also of assistance but not necessary. The shedding of *T. hyodysenteriae* in the feces is highly associated with the onset and recurrence of diarrhea; however, the latter may be sporadic and intermittent. The diarrhea of swine dysentery and the discharge of *T. hyodysenteriae* in the feces are probably associated with the periodic overgrowth and overpopulation of the organism in the crypts of the colon where it lives. The problem of diagnosing swine dysentery becomes more complicated when attempting to evaluate the carrier status of swine from suspected herds with a clinical history of swine dysentery but which do not have any clinical signs at the time of collecting rectal swabs. Nondiarrheic swine which are shedders of *T. hyodysenteriae* do exist, since 11.9% of the specimens producing strong beta-hemolytic reactions when cultured and 15.1% of the stained smears with large spirochetes came from swine with no diarrhea at the time of swabbing. The use of culturing and the isolation of *T. hyodysenteriae* may be of assistance in identifying endemic but nondiarrheic herds of swine suspected of being infected with swine dysentery. Certainly the staining of fecal smears should not be used alone in these herds because of the possibility that the presence of nonpathogenic large spirochetes could be mistaken for pathogenic large

spirochetes. Currently, one of us (L. D. O.) has under observation a group of swine with occasional soft stools and which shed large spirochetes; however, these spirochetes will not grow in culture. With the use of culturing in swine herds without clinical signs, the possibility exists of isolating *T. innocens* in attempting to recover *T. hyodysenteriae* and falsely diagnosing swine dysentery (Lysons et al., Proc. Int. Symp. Vet. Lab. Diag. 2nd, 1980) or not getting any growth and reporting an infected herd as being free of swine dysentery because the nondiarrheic carriers were not shedding *T. hyodysenteriae* at the time of swabbing (11). One of us (L. D. O.) has known of instances where these misdiagnoses of swine dysentery have occurred.

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