Experimental Infection with *Treponema hyodysenteriae* in Guinea Pigs

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Outbred and inbred (Hartley strain) guinea pigs (GP) were inoculated intragastrically with pathogenic and nonpathogenic *Treponema hyodysenteriae*. GP 3 to 16 weeks old received *T. hyodysenteriae* after a fasting period of 36 to 72 h. Infected GP with pathogenic *T. hyodysenteriae* developed a diarrheal and/or depressive condition, with mucus but not blood in the feces. Of 88 GP, 40 had gross lesions resembling those of swine dysentery. Lesions were limited mainly to the large intestine. GP used as controls or inoculated with nonpathogenic *T. hyodysenteriae* did not develop these lesions in the large intestine. These studies suggest that the GP may be used as an animal model for swine dysentery.

Swine dysentery (SD) is a mucohemorrhagic enteritis confined to the large intestines of swine (1, 6, 17). The disease affects swine of all ages and occurs throughout most swine-raising areas of the world (1, 7). The primary etiological agent of the disease is an anaerobic spirochete, *Treponema hyodysenteriae* (5, 7, 8, 16).

Attempts to produce the disease in guinea pigs (GP), rabbits, and mice through parenteral injections of *T. hyodysenteriae* have failed (10). In addition, oral inoculation of germfree and gnotobiotic mice colonized with Schaedler flora (eight nonpathogenic organisms) and with *T. hyodysenteriae* failed to produce the disease (R. D. Glock and D. L. Harris, unpublished data). Because GP had been used elsewhere for models of enteric diseases (2-4, 13), our efforts were directed at this laboratory animal.


**MATERIALS AND METHODS**

Pathogenic cultures. *T. hyodysenteriae* isolates B204, B169, B234 (supplied by J. M. Kinyon, Iowa State University, Ames, Iowa), and A-1 (supplied by D. J. Taylor, Cambridge University, Cambridge, England) were obtained from pigs affected with SD and shown to be pathogenic in pigs (12). Isolates B282 and B473 were reisolated from GP experimentally infected with *T. hyodysenteriae* isolates B204 and B234, respectively, and shown to be pathogenic in pigs (L. A. Joens, Ph.D. Thesis, Iowa State University, Ames, Iowa, 1977).

Nonpathogenic cultures. Isolates B256 (also supplied by J. M. Kinyon and B297 were obtained from pigs with signs of postweaning diarrhea. Isolate 4/71 was obtained in pure culture from D. J. Taylor who had isolated the spirochete from a normal pig. Puppy isolate was obtained from a puppy with cattarrhal enteritis. Puppy, B256, B297, and 4/71 isolates were nonpathogenic in pigs (12).

**Culture media.** Trypticase soy agar (TSA; Baltimore Biological Laboratory, Cockeysville, Md.) supplemented with 5% (vol/vol) citrated bovine blood (1 g of citrate per 100 ml of blood) was used for the isolation of *T. hyodysenteriae* by plating of dilutions and titrations of colonic material obtained from rectal swabs and mucosal scrapings and for viable count determination at time of GP inoculation (11). TSA supplemented with spectinomycin sulfate (Upjohn Co., Kalamazoo, Mich., 400 µg/ml, TSA-S400) was used for isolation of *T. hyodysenteriae* by direct culturing of rectal swabs and mucosal scrapings (15).

Two types of liquid media were used to cultivate *T. hyodysenteriae*. (i) Preduced anaerobically sterilized Trypticase soy broth (TSB; Baltimore Biological Laboratory, Cockeysville, Md.) was prepared as described by Holdeman and Moore (9) without cysteine or resazarin (PRAS-CF-TSB) (K. K. Kernstock, M.S. Thesis, Iowa State University, Ames, Iowa, 1976). (ii) TSB (aerobic-TSB) was prepared aerobically as described by Kinyon and Harris (11).

*T. hyodysenteriae* inocula. Inoculum was produced by culturing *T. hyodysenteriae* in tubed broth as described by Kinyon and Harris (11) and in flask broth. Cultures of *T. hyodysenteriae*, passaged less than 15 times, were subcultured (11%, vol/vol) into 500- and 2,000-ml round-bottomed flasks containing 180 and 1,000 ml of either aerobic-TSB or PRAS-CF-
TSB, respectively. The flask broth was supplemented with fetal calf serum (10%, vol/vol) and incubated under an atmosphere of 50:50 deoxygenated H2:CO2 for 24 to 36 h at 38°C with constant agitation on a reciprocating shaker (90 rpm). Concentrated cells of

T. hyodysenteriae (10x) were obtained by centrifugation of a log-phase culture at 10,000 x g at 4°C. The resulting pellet was resuspended in a volume of supernatant to equal a log increase in cells. Inoculum was checked for contaminating microorganisms at transfer by inoculating thioglycolate broth and by phase-contrast microscopy.

Source and care of GP. A total of 159 outbred GP (149, 2 to 4 weeks old, and 10, 16 weeks old) of mixed sexes were obtained from James Henderson, Cedar Falls, Iowa. Ten male inbred (Hartley strain) GP, 2 to 3 weeks old, were obtained from the colony at the National Animal Disease Center. Guinea pigs were housed in stainless steel isolation cages. The GP received a diet of oats and lettuce except during preinoculation fasting periods of 36 to 72 h.

GP Inoculation. The GP were inoculated with either culture or sterile TSB intragastrically via a 18-gauge, curved, blunt-end metal catheter and 20-ml syringe in 10-ml amounts for 2 to 3 consecutive days. Of 169 GP, 28 died at time of inoculation and were not included in the experimental data. The GP were checked daily for clinical signs of infection, which included diarrhea and depression (loss of appetite, ruffled coat, or listlessness).

Data collection. Postmortem examinations were performed at death or at the end of the 30-day study. Tissue sections of colons, ceca, and small intestines were collected and fixed in 10% buffered Formalin. Tissues were embedded and sectioned at 6 μm, mounted on glass slides, and stained with hematoxylin and eosin. Sections of the large and small intestines were also stained by the Warthin-Starry method. The stained tissue sections were examined by light microscopy for lesions and for the presence of spirochetes.

Isolation procedures for Salmonella. Colons, ceca, and small intestines from all GP were opened aseptically and sampled for Salmonella. Mucosal scrapings of each tissue were placed in 20 ml of tetrahionate broth (~1 g) and streaked directly on Tergitol-7 agar. Subcultures onto brilliant green agar from tetrahionate broth were made after 24 h of incubation at 37°C. Lactose-negative colonies were identified as described by Oetjen and Harris (14). Suspected Salmonella colonies were screened with polyvalent "O" antisera (Difco Laboratories). Positive colonies were then serotyped (B. Blackburn, Diagnostic Services, Animal and Plant Health Inspection Services, National Veterinary Services Laboratory, Ames, Iowa).

RESULTS

Inoculation of GP with pathogenic cultures. Clinical signs of disease, either depression and/or diarrhea, were observed in 23 of 88 GP at 2 to 8 days postinoculation. Of 88 GP, 47 died during the experimental period. Of 88, 40 GP had macroscopic lesions similar to those in pigs with SD. Macroscopic lesions of the cecum and occasionally the colon varied from catarrhal inflammation to hemorrhage; lumen contents ranged from soft, normal debris to bloody, watery exudate. Occasional edema and catarrhal inflammation were seen in the small intestine. Hyperemia and distention were noted of the serosa in GP with hemorrhagic inflammation of the mucosa.

Of 88 mucosal scrapings of GP cecal tissue, 36 were positive for T. hyodysenteriae by phase microscopy. Of 23 GP, 9 were positive for T. hyodysenteriae by plating dilutions and filtrations of colonic material on TSA. Of 65 GP, 30 were positive for T. hyodysenteriae by plating of colonic material on TSA-S400 (Table 1). No clinical signs or lesions were noted in 16-week-old GP inoculated with isolate B204 nor in 3- to 4-week-old GP inoculated with isolate B282.

Microscopically, the crypts appeared to have numerous T. hyodysenteriae present with some goblet cell hyperplasia. The blood vessels of the lamina propria appeared to be congested in some GP. Areas of hemorrhage and edema were seen in the mucosa, and in some cases complete erosion of the mucosal surface was noted. Leukocytic infiltration was seen in the lamina propria, consisting mainly of neutrophils and macrophages.

Inoculation of GP with nonpathogenic cultures. One GP inoculated with puppy isolate had soft to loose fecal material but not diarrhea at time of necropsy. Gross lesions were not detected in the intestines or ceca of GP inoculated with nonpathogenic isolates. Of 19 inoculated GP, 9 died during the experimental period.

Five of 19 mucosal scrapings of GP cecal tissue were positive for T. hyodysenteriae by phase microscopy. Three of 19 GP were positive for T. hyodysenteriae by culture on TSA-S400; however, the zone of beta-hemolysis produced by the spirochete on blood agar was characteristic of that produced by nonpathogenic isolates (Table 2).

Inoculation of controls with sterile TSB. No clinical signs of depression or diarrhea were seen in the group inoculated with sterile TSB during the challenge experiment. No gross lesions were noted. Microscopically, the large intestines appeared normal except for some autolysis believed to be due to postmortem change. Of 34 GP, 18 died during the experimental period (Table 2).

Salmonella spp. (serotype 4, 12:eh-mpo-

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TABLE 1. Results of intragastric inoculation of GP with pathogenic isolates of T. hyodysenteriae

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. of GP</th>
<th>Inoculum* titer</th>
<th>(10^6)</th>
<th>Depression</th>
<th>Diarrhea</th>
<th>Gross lesions</th>
<th>Phase microscopy</th>
<th>Culture</th>
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<tbody>
<tr>
<td>B204</td>
<td>10(^c)</td>
<td>(5.2 \times 10^6)</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1(^d)</td>
<td></td>
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<tr>
<td>B204</td>
<td>3</td>
<td>(2.2 \times 10^6)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1(^e)</td>
<td></td>
</tr>
<tr>
<td>B282</td>
<td>5</td>
<td>(6.0 \times 10^6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B169</td>
<td>5</td>
<td>(2.6 \times 10^{10})</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2(^f)</td>
<td></td>
</tr>
<tr>
<td>B234</td>
<td>24</td>
<td>(2.6 \times 10^6)</td>
<td>6</td>
<td>0</td>
<td>12</td>
<td>10</td>
<td>13(^g)</td>
<td></td>
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<tr>
<td>B234</td>
<td>25</td>
<td>(2.9 \times 10^9)</td>
<td>7</td>
<td>4</td>
<td>14</td>
<td>13</td>
<td>14(^g)</td>
<td></td>
</tr>
<tr>
<td>B473</td>
<td>4(^i)</td>
<td>(2.0 \times 10^6)</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3(^j)</td>
<td></td>
</tr>
<tr>
<td>A-1</td>
<td>4</td>
<td>(6.3 \times 10^6)</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1(^k)</td>
<td></td>
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<tr>
<td>A-1</td>
<td>8</td>
<td>(6.6 \times 10^{10})</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>4(^l)</td>
<td></td>
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</table>

* Geometric mean value of the number of T. hyodysenteriae given per GP.
\(b\) Number of GP showing signs of depression and diarrhea.
\(c\) These numbers include four outbred GP 16 weeks of age.
\(d\) Isolation of T. hyodysenteriae through plating of dilutions and titrations of colonic material on TSA.
\(e\) Isolation of T. hyodysenteriae by direct plating of colonic material on TSA-S400.
\(f\) Number of inbred (Hartley strain) GP inoculated with isolated B473.

TABLE 2. Results of intragastric inoculation of GP with nonpathogenic isolates of T. hyodysenteriae and TSB

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. of GP</th>
<th>Inoculum* titer</th>
<th>(10^6)</th>
<th>Depression</th>
<th>Diarrhea</th>
<th>Gross lesions</th>
<th>Phase microscopy</th>
<th>Culture</th>
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<tbody>
<tr>
<td>B256</td>
<td>5</td>
<td>(3.0 \times 10^6)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B297</td>
<td>5</td>
<td>(6.2 \times 10^6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4/71</td>
<td>5</td>
<td>(3.2 \times 10^6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Puppy</td>
<td>4</td>
<td>(4.4 \times 10^6)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td></td>
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<tr>
<td>TSB (Controls)</td>
<td>34(^d)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Geometric mean value of the number of T. hyodysenteriae given per GP.
\(b\) Number of GP showing signs of depression and diarrhea.
\(c\) Isolation of nonpathogenic T. hyodysenteriae by direct plating of colonic material on TSA-S400.
\(d\) These numbers include five inbred (Hartley strain) GP and four outbred GP (16 weeks of age) used as controls.

GP negative for gross lesions. Inbred GP were negative for Salmonella.

Coccidia were detected microscopically in the cecal tissues of four outbred GP with lesions similar to those of SD. This organism was not present in the tissues of inbred GP.

DISCUSSION

Results in this report confirm our earlier work on the production of lesions similar to those of SD in the GP. Diarrhea and/or depression was noted in GP inoculated with pathogenic T. hyodysenteriae, except in GP inoculated with isolate B282 and in 16-week-old GP inoculated with isolate B204.

Gross lesions were produced in GP inoculated with isolates B204, B234, B473, and A-1. The lesions were especially pronounced in GP inoculated with isolate B234. A good correlation between phase microscopy and culture isolation of the spirochete was demonstrated in inoculated GP.

The histopathological examinations of infected cecal and colonic tissue revealed characteristic lesions similar to those in SD. Also, the crypts of infected GP contained numerous spirochetes. Isolates of T. hyodysenteriae that were nonpathogenic for swine failed to produce either macroscopic or microscopic lesions in the intestines of inoculated GP. Of four GP inoculated with the puppy isolate, three appeared to be infected with T. hyodysenteriae; however, no evidence of disease was noted.

Deaths of GP during inoculation were due to complications after esophageal puncture or due to suffocation by obstruction of the air passage with the inoculum. Deaths of GP after inoculation were attributed to infection, the stress of the preinoculation fast and other intrinsic factors undetected by observation or necropsy. None of the inbred GP that served as controls died.

Apparently, Salmonella spp. (serotype 4, 12:eh-monophasic) was endemic in the outbred...
GP colony used as the source of animals for part of this study. The effect of this serotype in production of intestinal lesions in GP by T. hyodysenteriae is not known. However, the salmonella serotype was present in GP with and without intestinal lesions. Also, the deep necrotic involvement in the submucosa usually associated with salmonellosis was not seen. The above evidence and the production of lesions typical of SD in salmonella-free inbred GP argue against involvement of Salmonella spp. (serotype 4, 12:eh-monophasic) in the production of intestinal lesions.

Coccidia were detected in four outbred GP infected with T. hyodysenteriae. However, this protozoan did not appear to contribute to the experimental disease; the lesions produced by T. hyodysenteriae in the intestinal tissues of GP with coccidia were indistinguishable from those produced in GP tissues infected with T. hyodysenteriae and free of coccidia.

We observed several similarities and differences between GP and swine infected with T. hyodysenteriae. The similarities were as follows: (i) the infection was completely localized to the large intestine; (ii) diarrhea was present; (iii) gross lesions in the large intestine ranged from catarrhal to hemorrhagic inflammation of the mucosa; and (iv) microscopic lesions consisted of cellular infiltration of the lamina propria, erosion of the epithelial surface, and necrosis and edema.

The differences observed were as follows: (i) in many GP, lesions similar to SD were found only in the cecum; (ii) diarrhea with blood and/or fibrin was not detected; and (iii) clinical signs appeared earlier.

We propose that the GP may serve as an animal model for this disease which could reduce the cost of animal experimentations and the need for animal space and caretaker personnel and which may provide researchers with an in vivo means for immunological and pathogenicity studies on T. hyodysenteriae.

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LITERATURE CITED