

PATHOGENICITY OF *TREPONEMA HYODYSENTERIAE* IN UNCONTAMINATED GNOTOBIOTIC PIGS

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Swine dysentery has been induced in conventional pigs by inoculation with *Treponema hyodysenteriae* (*T. hyo.*) but not in uncontaminated gnotobiotic pigs. When colitis was induced in gnotobiotic pigs with *T. hyo.* in combination with 4 obligate anaerobes, Whipp et al. hypothesized that the etiology of swine dysentery involved a mixed infection. Support for this concept was provided by our observations that: (1) colitis occurred in gnotobiotic pigs inoculated with *T. hyo.* in combination with any one of 7 different anaerobes; (2) colitis was unobserved in uncontaminated gnotobiotic pigs infected with *T. hyo.* alone. Our repeated failure to recover *T. hyo.* from gnotobiotic pigs infected with *T. hyo.* alone led us to hypothesize that the synergistic relationship between *T. hyo.* and certain other anaerobes was related to colonization and not to expression of pathogenicity. The objectives of this study were to: (1) facilitate colonization of the colon of gnotobiotic pigs by *T. hyo.* with frequent injections of growth media directly into the cecum; (2) to test the hypothesis that *T. hyo.* was present in pigs inoculated with *T. hyo.* only, but in numbers below the sensitivity of our culture methods; and (3) to test 2 other isolates for synergism with *T. hyo.*

## Methods:

Gnotobiotic pigs were derived as described by Miniats et al. In the first set of experiments, pigs were maintained as previously except that a cannula was implanted into the cecum of some pigs during the first week of life to facilitate injection of media. Of 38 pigs inoculated with *T. hyo.* alone or in various combinations with other anaerobes and/or media in the cecum, the results from 27 were invalidated by incidental contamination.

In the second series of experiments, the diet was autoclaved for 15 min since we had demonstrated this to be one source of incidental contamination. In these experiments, pigs were inoculated with the various anaerobes during the first week of life and with *T. hyo.* during the second week of life. The microorganisms used included *Fusobacterium plauti*, which was isolated from the colonic mucosa of conventional pigs with experimental swine dysentery and a ureolytic *Streptococcus* spp. isolated from the colonic mucosa of a conventional clinically-normal pig. The numbers of pigs and inocula used in these experiments are given in Tables 1 and 2. Bacterial contamination was unobserved in these pigs.

TABLE 1

| No. of pigs | Inoculum                          | <i>T. hyo.</i> recovered | Colitis observed |
|-------------|-----------------------------------|--------------------------|------------------|
| 3           | <i>T. hyo.</i> , <i>F. necro.</i> | 3/3                      | 3/3              |
| 1           | <i>T. hyo.</i> , <i>F. plauti</i> | 0/2                      | 0/2              |
| 1           | <i>T. hyo.</i> alone              | 2/2                      | 1/2              |

## Results:

Of the 11 uncontaminated pigs in the first series of experiments, *T. hyo.* induced colitis in 2 of 3 pigs which were pre-inoculated with *Bacteroides vulgatus* (*B. vul.*) and *Fusobacterium necrophorum* (*F. necro.*), but not in any of 3 pigs infected with *T. hyo.* alone, and *T. hyo.* was not recovered from these latter pigs. In 1 of 2 pigs inoculated with *B. vul.* 5 days after inoculation with *T. hyo.* only, colitis was observed and *T. hyo.* was recovered at necropsy. This suggests that failure to reisolate *T. hyo.* from some pigs

inoculated with *T. hyo.* only may be a function of the sensitivity of the culture techniques. *T. hyo.* was re-isolated 5 days after inoculation with *T. hyo.* only from 2 of 3 pigs in which growth media was injected every 6 h after inoculation, but colitis was unobserved. This demonstrates that the colonic environment can be altered to facilitate growth of *T. hyo.*

TABLE 2

| No. of pigs | Inoculum   | <i>T. hyo.</i> recovered | Colitis observed |
|-------------|--|--------------------------|------------------|
| 2           | <i>T. hyo.</i> , <i>F. necro.</i>                    | 1/2                      | 1/2              |
| 3           | <i>T. hyo.</i> , <i>F. necro.</i> ,<br><i>Strep.</i> | 3/3                      | 3/3              |
| 4           | <i>T. hyo.</i> , <i>Strep.</i>                       | 1/4                      | 0/4              |
| 4           | <i>T. hyo.</i> alone                                 | 2/4                      | 3/4              |

In the first experiment of the second series (Table 1), *T. hyo.* consistently induced colitis when present with *F. necro.*, but not when present with *F. plauti*, nor was *T. hyo.* recovered from pigs infected with *F. plauti*. *F. plauti* was consistently recovered from these pigs. The startling result was recovery of *T. hyo.* from 2 pigs infected with *T. hyo.* only and the presence of colitis in one of these pigs.

In the second experiment (Table 2), *T. hyo.* consistently induced colitis when present with *F. necro.*, but not when present with *Streptococcus*, although *T. hyo.* was recovered from 1 of 4 pigs infected with *T. hyo.* in combination with *Streptococcus*. *Streptococcus* was consistently isolated from pigs in which it had been inoculated. However, the results of the previous experiment were confirmed when *T. hyo.* was recovered from pigs inoculated with *T. hyo.* only and colitis was observed in some of these pigs. In both of these experiments, *T. hyo.* was shown by transmission electron microscopy, scanning electron microscopy or histology (Warthin-Starry stain) to inhabit the colonic mucosa in all animals positive for *T. hyo.* *T. hyo.* was observed more frequently in crypts than on the surface epithelium, but was never observed in subepithelial layers and did not appear to adhere to the epithelium. By SEM, they appeared to be incorporated into mucus at the crypt openings and deep in crypt lumina.

Thus, we have demonstrated that *T. hyo.* can colonize and express pathogenicity in the colon of the gnotobiotic pig in absence of other microbial contamination. We interpret this to provide additional support for the hypothesis that the synergism observed between *T. hyo.* and certain other anaerobes facilitates colonization and not expression of pathogenicity. The only procedural changes of which we are aware that may explain the difference between these results and previous observations are: (1) the autoclaved diet, and (2) subtle changes in growth media for *T. hyo.* resulting in a 10-fold increase in the number of cells in the inoculum.

Selected references: Harris, D.L., Alexander, T.J.L., Whipp, S.C., Robinson, I.M., Glock, R.D. and Matthews P.J.: J. Am. Vet. Med. Assoc. 1978, 122:468; Meyer, R.C., Simon, J. and Byerly, C.S.: Vet. Path. 1975, 12: 46; Whipp, S.C., Robinson, I.M., Harris, D.L., Glock, R.D., Matthews, P.J. and Alexander, T.J.L.: Infect. Immun. 1979, 26:1042; Whipp, S.C., Robinson, I.M., Harris, D.L., Glock, R.D. and Matthews, P.J.: Proc. IPVS 1980, p. 230; Miniats, O.P. and Jol, D.: Can. J. Comp. Med. 1978, 42:428.