Synergism Between *Trichurus suis* and the Microbial Flora of the Large Intestine Causing Dysentery in Pigs

J. M. RUTTER and R. J. S. BEER

Agricultural Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berkshire, England

Received for publication 2 October 1974

The role of the microbial flora of the large intestine in experimental *Trichurus suis* infection was studied by comparing the clinical syndrome in conventionally reared (CR) pigs, specific pathogen-free pigs, and gnotobiotic pigs. The disease in CR pigs was characterized by a severe mucohemorrhagic enteritis; in contrast, a mild catarrhal enteritis was observed in specific pathogen-free and gnotobiotic pigs. Spirochaetes and vibrio-like organisms were observed only in CR pigs and increased during the clinical phase of the disease. The clinical syndrome was not transmitted by oral administration of intestinal or fecal material from infected CR pigs to CR pigs free of *T. suis*. Smaller numbers of *T. suis* produced diarrhea in CR pigs and significantly reduced the growth rates of infected animals; clinical signs and the reduction in growth rate was prevented by incorporating an antibacterial substance (dimetridazole) in the food. Although clinical trichuriasis closely resembles swine dysentery, the two syndromes seem to be distinct. The present results suggest that a microbial component acts synergistically with *T. suis* to produce the severe clinical syndrome in CR pigs, but identification of the microbial component and the mechanism by which clinical signs are produced await further studies of the bacterial flora of the large intestine of pigs.

The nematode *Trichurus suis* is present in fattening pigs throughout the world (8) and its widespread occurrence, usually in light infections, has given the impression that the parasite presents no serious problem to animal health. However, severe outbreaks of trichuriasis associated with high mortality have been recognised in the U.S.S.R. in Europe and in the U.S.A. (5). Natural and experimental infections of weaned pigs with *T. suis* causes a severe mucohemorrhagic diarrhea and death (6, 7, 21, 27), and this syndrome closely resembles clinical descriptions of the disease termed swine dysentery (1, 18, 36). Swine dysentery causes serious losses to the pig industry and there have been numerous attempts to define its etiology. At first, *Vibrio coli* was thought to be responsible (14, 23, 26, 28), but several workers have been unable to reproduce the disease with cultures of *V. coli* (2, 3, 13). More recently, a spirochaete has been incriminated in swine dysentery (33, 35), and the disease can now be reproduced in pigs by feeding cultures of a large spirochaete classified as type 1 (32) or *Treponema hydysenteriae* (19). Spirochaetes have been observed invading within and between the cells of the colonic epithelium of pigs with swine dysentery (18, 32), and Beer and Rutter (7) reported that large spirochetes, apparently morphologically distinct from *T. hydysenteriae*, had invaded the mucosa of the large intestine of pigs experimentally infected with *T. suis*; these results suggested that the migrating *T. suis* larvae may damage the mucosal epithelium of the large intestine and allow penetration of microorganisms that contribute to the clinical signs that follow *T. suis* infection. The present study was part of an investigation of the role of bacterial infection in the clinical syndrome that follows experimental *T. suis* infection. In the first three experiments, the syndrome was compared in conventionally reared (CR), specific pathogen-free (SPF), and gnotobiotic pigs; in the subsequent experiments the effect of an antibacterial substance on *T. suis* infection in CR pigs was investigated.

MATERIALS AND METHODS

**Animals.** Trichuris-free, CR piglets from a herd with no history of swine dysentery were weighed and assigned to different groups using random numbers so that the total weight of each group was approximately equal. Each group was housed in separate pens in the same building. The SPF pigs were second generation...
stock from parents that were derived by hysterotomy; the SPF pigs were free from enteric disease and were housed in a separate building. In the first three experiments, CR and SPF pigs were fed Vitasukla 17 (Vitamealo, Beecham Agricultural Products); in subsequent experiments, CR pigs were fed dry meal containing 50% barley, 15% wheat, 20% wheatings, and 15% proprietary concentrate ad libitum. Water was provided ad libitum for all animals. The piglets were weaned immediately prior to infection with T. suis. Gnotobiotic piglets were derived and maintained in plastic isolators (30, 34).

**Infection procedure.** Infective T. suis ova were suspended in saline and given to the piglets by stomach tube. The procedures for extraction of eggs from feces and culture to the infective stage have been described (5a).

**Clinical observations and weight gains.** All pigs were examined daily for the onset of clinical signs and weighed at frequent intervals.

**Necropsy procedures.** Pigs were slaughtered by immobilization with etorphine hydrochloride and acepromazine maleate (Immobilon, Reckitt and Coleman), followed by intracardiac injection of pentobarbital sodium (Euthatal, Abbott Laboratories) and exsanguination. The abdomen was opened, the intestines were removed, and the mesentery was cut so that the large intestine could be divided into three equal parts. The large intestine was scraped to remove the mucosa from the underlying submucosal layer, then the scrapings were digested and the number of T. suis were counted (7).

**Microscopy examinations.** Fecal samples and intestinal contents were diluted and examined (7).

**Bacteriological procedures.** Rectal swabs were cultured on bovine blood agar, MacConkey agar, and brilliant green agar and incubated aerobically at 37 C, or on neomycin bovine blood agar and DeMan, Rogosa and Sharpe agar incubated anaerobically in anaerobic jars (Baird and Tatlock Ltd). Fecal samples and intestinal contents were diluted 1:2 (wt/vol) in saline and filtered through a 0.65-nm membrane filter (Millipore Corp.) and then the filtrate was cultured on bovine blood agar or dropped onto a 0.22-nm membrane filter disk (Millipore Corp.) placed on the plate. Cultures were incubated anaerobically for at least 4 days.

**Histological procedures.** Tissues removed at necropsy were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned 5-nm intervals, and stained by hematoxylin and eosin, Gram, Giemsa, or Warthin-Faulkner stain (11).

**RESULTS**

Experiment 1: (i) experimental T. suis infection in CR pigs. Nine pigs were each infected with 100,000 T. suis ova and 10 pigs were left as uninfected controls. The infected pigs began passing loose feces after 10 days, and by day 18 several pigs had lost their appetite, had diarrhea, and were depressed. By day 26, the infected pigs were emaciated and had profuse diarrhea, which in most cases contained fresh blood and large quantities of mucus. The uninfected pigs showed no clinical signs. The cumulative totals of the mean changes in body weight of the pigs are shown in Fig. 1. During the first 14 days after infection, the control pigs grew faster than the infected group; after 14 days, growth of the infected pigs was reduced and the pigs lost weight from day 20 until they were killed.

(ii) Necropsy lesions. A progressive enteritis was present in the infected pigs; this was initially catarrhal but later became mucohemorrhagic. Fourteen days after infection the wall of the large intestine was thickened and edematous, and the lumen contained large quantities of clear mucus. After 20 days, the intestinal contents contained large quantities of mucus, fresh blood, and necrotic debris that separated from the underlying mucosa. In some cases, the lesion progressed to become a thick, necrotic pseudomembrane that covered the mucosal surface of the large intestine. No lesions were observed in the uninfected animals. The histopathological results will be described in a separate communication.

(iii) Microbiology. Large numbers of T. suis were recovered from the large intestines of infected pigs (range 14,200 to 53,500, mean 32,500). Bacterial cultures of fecal samples from all pigs before infection with T. suis yielded predominantly coliform bacteria, gram-negative anaerobic bacteria, lactobacilli, and streptococci; small numbers of vibrio-like organisms (VLO) and spirochetes of different morphology were present in some of the pigs. Similar

![Growth Rate of Conventionally Reared Pigs Infected with Large Numbers of Trichuris suis](image-url)
bacteria were present after *T. suis* infection, and from 12 days, increased numbers of VLO and spirochetes were detected by phase contrast microscopy in fecal samples from the infected pigs. Increased numbers of these organisms were present in mucosal scrapings from the large intestine of infected compared with control pigs; both VLO and spirochetes increased in some infected pigs, but in others, spirochetes or VLO increased.

(iv) **Microscopy.** In sections stained by the Warthin-Faulkner method, the crypts of the large intestine of uninfected pigs frequently contained small rod-shaped or curved bacteria. From 15 days after infection, many organisms were present in the excessive mucous secretions on the surface of the large intestine of infected pigs compared with the controls; the bacteria were numerous adjacent to *T. suis* larvae (Fig. 2) and did not stain by Gram’s method. Few bacteria were present in the crypts of the infected pigs.

A few organisms were seen adjacent to the mucosal epithelium in sections of the large intestine of control pigs by electron microscopy, but large numbers of bacteria were present in infected pigs adjacent to *T. suis* larvae (Fig. 3); these included rod-shaped or curved bacteria approximately 400 nm in diameter and up to 1.5 \( \mu \text{m} \) long. Few spirochetes were seen and there was little evidence of microbial invasion of the surface epithelium.

**Experiment 2: attempts to transmit the clinical syndrome to pigs free of *T. suis.*** Diarrheic feces containing blood and mucus were collected from pigs 18 days after infection with *T. suis*; the material was diluted in an equal volume of phosphate-buffered saline and 50 ml was given by stomach tube to each of six weaned pigs. Four days later, each pig was given 20 ml of material collected from pigs with clinical disease 22 days after infection with *T. suis*. After a further 7 days, each pig was given 20 ml of material containing blood, mucus, and desquamated epithelium collected from the large intestine of a pig that was killed when moribund 23 days after *T. suis* infection. No clinical signs were observed after dosing with these preparations. Infective *T. suis* ova had not developed in the donor pigs and we concluded that the bacteria alone could not initiate the disease syndrome.

![Fig. 2. Bacteria (B) proliferating adjacent to a *T. suis* larva (L) in the large intestine of a CR pig killed 27 days after infection. Warthin-Faulkner stain. ×753.](image)
Experiment 3: (i) experimental *T. suis* infection in SPF pigs. Six pigs were infected with 100,000 *T. suis* ova and five pigs were left as uninfected controls. One infected pig passed loose feces intermittently between 19 and 26 days after infection; otherwise there were no clinical signs. The cumulative totals of the mean changes in body weight are shown in Fig. 4. The uninfected pigs grew slightly better than the infected pigs throughout the experiment and no loss in weight occurred in the infected pigs.

(ii) Necropsy lesions. Fifteen days after infection, the wall of the large intestine of

*Fig. 3.* Bacteria proliferating adjacent to the epithelium of the large intestine of a CR pig killed 25 days after infection with *T. suis*. ×23,700.
infected pigs was thickened and edematous, and by day 20, excessive mucous production and small petechial hemorrhages were present. After 23 days, foci of mucosa covered by an exudate of mucus and necrotic debris were observed in the upper third of the large intestine. No lesions were found in the uninected animals.

(iii) Microbiology. Large numbers of T. suis were recovered from the large intestines of infected pigs (range 11,600 to 50,800, mean 28,000). Before infection with T. suis, fecal samples yielded coliform bacteria, gram-negative anaerobic bacteria, lactobacilli, and streptococci; similar bacteria were recovered after infection with T. suis and, in contrast to the CR pigs, no VLO or spirochetes were observed.

(iv) Microscopy. A few small rods of coccobacilli were present on the mucosal surface of the large intestine and in the crypts of Lieberkühn of the control piglets. In contrast, increased numbers of bacteria were present in the excessive mucous secretions of infected pigs from 15 days after infection; in general, there were fewer organisms than in infected CR pigs. The bacteria varied in size from long rods to small coccobacilli, and in Gram-stained sections, there were more gram-positive bacilli and coccobacilli than in the infected CR pigs. Increased numbers of organisms were seen in sections of the large intestine of infected pigs by electron microscopy; these were sometimes present in vacuoles and were generally close to T. suis larvae. The organisms were rod shaped, approximately 440 nm in diameter, and up to 2 μm long (Fig. 5); granules were present in the bacterial cytoplasm, and the organisms appeared to be morphologically different from those proliferating in CR pigs.

Experiment 4: experimental T. suis infection in gnotobiotic piglets. Preliminary experiments indicated that T. suis ova did not hatch in the large intestine of germfree piglets but could be recovered intact from the slurry. Pairs of 7-day-old germfree piglets were then infected with a strain of Staphylococcus aureus, a nonhemolytic strain of Escherichia coli, or a strain of Lactobacillus spp. all isolated from CR pigs. The piglets were given 100,000 T. suis ova 4 days later and, after a further 5 days, the slurry was collected and the percentage of hatch of T. suis ova was calculated by examining the slurry under the microscope. The hatch was 0, 12, and 30%, respectively, indicating that colonization of the intestine of germfree pigs with a Lactobacillus spp. gave the best hatch of T. suis ova.

Four 7-day-old gnotobiotic piglets were then colonized with the Lactobacillus spp., and after 5 days each piglet was given 100,000 T. suis ova. A piglet was killed 15, 22, 25, and 30 days after infection with T. suis. No diarrhea was observed during this period, and at necropsy the lesions were confined to the cecum and first half of the large intestine; initially, the intestinal wall became thickened and, later, excessive clear mucus was present in the intestinal lumen. No petechial hemorrhages or necrotic debris were observed. The numbers of T. suis recovered were 1,049 to 9,740 (average 4,160).

Experiment 5: effect of dimetridazole on severe clinical trichuriasis in CR pigs. Two groups of seven pigs (groups 1 and 2) were each given 100,000 T. suis ova and eight pigs were left as uninfected controls (group 3). Group 1 received 0.05% (wt/wt) dimetridazole (1,2-dimethyl-5-nitroimidazole, 8595 RP, Emtryl, May and Baker Ltd.) as a food additive from day 13, when clinical signs developed, to the end of the experiment. The development of clinical signs was delayed in group 1, but by day 25, most of the pigs in groups 1 and 2 had typical signs of T. suis infection. The pigs were slaughtered 32 days after infection. Large numbers of T. suis were recovered from group 1 (range 1,600 to 36,200, mean 22,800) and group 2 (range 200 to 36,400, mean 13,800). At necropsy, the lesions in the large intestine of pigs in group 1 were less severe than those of group 2. In general, the degree of damage to the large intestine was related to the numbers of T. suis...
present; however, in some cases, there was considerable damage and yet only small numbers of *T. suis* were recovered.

**Experiment 6:** (i) effect of dimetridazole on mild clinical trichuriasis in CR pigs. Two groups of eight pigs were given 70,000 *T. suis* ova (groups 1 and 2) and eight pigs were left as uninfected controls (group 3). Group 1 was given 0.05% (wt/wt) dimetridazole as a food additive throughout the experiment. Clinical signs were confined to the infected, untreated pigs (group 2), which began passing loose feces 16 days after infection. By day 25 all pigs in group 2 had diarrhea, but this contained no

**Fig. 5.** Bacteria proliferating in vesicles in the large intestine of a SPF pig killed 35 days after infection with *T. suis*. ×33,600.
blood or mucus. The pigs were slaughtered 32 days after infection.

The cumulative totals of the mean changes in body weight of the three groups were analyzed by a split-plot analysis, with the three groups as the main plot and the number of days after infection as the subplot. There was no significant difference between the totals of mean changes in body weight of groups 1 (infected, treated) and 3 (uninfected) during the experiment. By day 20, the cumulative total of mean changes in body weight of group 2 (infected, untreated) was significantly lower than group 3 ($P < 0.01$), and by day 24, the cumulative total of mean changes in body weight of group 2 was significantly lower than group 1 ($P < 0.01$); these differences continued to the end of the experiment.

(ii) Necropsy findings. Necropsy lesions were confined to groups 1 and 2. The mucosa of the large intestine of the majority of pigs from group 2 was hyperemic, thickened, and edematous with petechial hemorrhages and excessive mucous production. The lesions in group 1 were less severe compared with group 2 and fewer pigs were affected.

(iii) Microbiology. *T. suis* was recovered from group 1 (range 2,700 to 11,300, mean 6,700) and group 2 (range 5,100 to 11,600, mean 8,600). The numbers of spirochetes and VLO observed in wet preparations from fecal material and in sections stained by the Warthin-Faulkner method were greater in group 2 compared with group 1.

(iv) Microscopy. In general, there were more bacteria in the mucous exudate of the large intestine in pigs from group 2 compared with group 1. Bacteria, including spirochetes, were seen proliferating close to *T. suis* larvae in sections of the large intestine of pigs from group 2 by electron microscopy (Fig. 6), but few organisms were seen in sections from the other pigs.

**DISCUSSION**

The clinical syndrome and necropsy lesions of *T. suis* infection are similar to those described for swine dysentery; in both diseases there is a profuse mucohemorrhagic diarrhea, the lesions are confined to the large intestine, and natural infection is most severe in young weaned pigs. Although these similarities raise the possibility that swine dysentery is initiated by *T. suis* infection, this interpretation seems unlikely for several reasons: severe clinical signs with *T. suis* infection generally require the presence of large numbers of the nematode; and although *T. suis* is widely distributed in fattening pigs throughout the world (8), there is little evidence that it reaches sufficiently high numbers (9) to account for the widespread occurrence of swine dysentery. Furthermore, swine dysentery is characterized by the presence of large numbers of *Treponema hyodysenteriae* in the large intestine of infected pigs and the disease can be reproduced by oral administration of the spirochete (21, 31). Although the present results indicate that increased numbers of spirochetes can occur in pigs with experimental *T. suis* infection, we were unable to transmit the disease by feeding intestinal material from infected pigs to pigs free of *T. suis*. Thus, swine dysentery and experimental *T. suis* infection appear to be different syndromes, and the similar clinical signs may reflect a restricted capacity of the large intestine to respond to injury. These conclusions do not exclude the possibility that some outbreaks of swine dysentery may be attributable to *T. suis* infection, and a closer examination of such outbreaks in pigs housed in conditions that would favor *T. suis* seems justified.

The results of the present experiments suggest that a component of the bacterial flora of the large intestine plays a significant role in the clinical syndrome of experimental *T. suis* infection. CR pigs infected with large numbers of the nematode became severely diseased, whereas SPF pigs with similar burdens of the nematode showed virtually no clinical signs. Necropsy lesions in SPF pigs indicated that *T. suis* larvae produce thickening of the wall of the large intestine and excessive mucous production; a similar lesion was present in the early stages of infection in CR pigs, but by 20 days, there were hemorrhages and necrosis. During this period, large numbers of bacteria had proliferated in the exudate and the pigs stopped eating and growing. Bacteria also proliferated in the exudate of SPF pigs infected with *T. suis*; therefore, it seems that different types of bacteria may multiply in the two groups and that the postulated microbial component was absent from the SPF pigs. Because disease did not occur in the SPF pigs, we concluded that the microbial component is not carried within the nematode ova.

The results of *T. suis* infection in gnotobiotic piglets confirmed that in the absence of a conventional gut flora, *T. suis* larvae produce thickening of the gut wall and excessive mucous secretion. The failure of *T. suis* ova to hatch in germfree piglets is presumably related to the gut environment. Batte and Moncol (4) stated
that infective ova of *T. suis* are softened by stomach secretions so that the larvae are liberated; the pH of the stomach contents of germ-free piglets may be less acid than that of gnotobiotic piglets contaminated with lactobacilli, which may account for the better hatch obtained in the latter group.

If bacteria contribute significantly to the clinical syndrome of *T. suis* infection, then it should be possible to modify the development of the disease with antibacterial substances. Support for this view was obtained by incorporating dimetridazole in the feed of CR piglets with mild trichuriasis; this prevented the develop-
ment of diarrhea and enabled the treated piglets to grow as well as the control group. However, dimetridazole was not effective in CR pigs with severe trichuriasis; this may have been attributable to reduced intake of the drug as a result of anorexia. These results are consistent with the view that a microbial component in CR pigs acts synergistically with *T. suis* to produce the clinical signs of infection. Microbial synergism can be defined as the combined activity of two or more microbial agents that separately produce minimal lesions but together cause a more severe reaction than would be expected from a summation of the individual effects. *T. suis* produces only a catarrhal enteritis; the microbial component in CR pigs cannot initiate the disease in the absence of *T. suis*, and it is only when the two agents are present together that the severe clinical syndrome occurs.

The clinical signs caused by *T. suis* infection begin about 12 days after administration of the drug; this corresponds with the time at which the larvae protrude their posterior tips through the mucosal surface with localized rupture of the epithelial cells. Presumably, the emerging larvae stimulate the production of excessive mucus in which the microbial component of CR pigs multiplies. We have not identified this microbial component or the mechanism by which clinical signs are produced in the present study, but the following observations are worthy of mention: (i) we were unable to demonstrate VLO or spirochetes in SPF pigs, and (ii) VLO and spirochetes increased in infected pigs with clinical signs and were reduced in infected pigs treated with dimetridazole. Bacterial proliferation occurred predominantly in the mucous exudate, and invasion of the mucosa did not appear to be a significant component of the lesion. Dimetridazole has been effective in controlling swine dysentery; it has antimicrobial activity in vitro, against spirochetes, vibrios, fusiforms, *Bacteroides*, and *Clostridia* spp. (17); although dimetridazole has anti-protozoal activity (17), the recoveries of *T. suis* from groups 1 and 2 in experiments 5 and 6 suggest that the drug has no activity against the nematode. Since spirochetes were not demonstrated in all the infected pigs during the present study, it seems likely that other organisms, or combination of organisms, produce the clinical signs of *T. suis* infection, and further studies are necessary to identify the microbial flora of the large intestine of CR and SPF pigs.

An early lesion in the pathogenesis of swine dysentery is catarrhal enteritis (18), and since the disease is not produced in germfree pigs with *T. hyodysenteriae*, it has now been suggested that synergism between *T. hyodysenteriae* and a portion of the normal enteric flora occurs in swine dysentery (16). As in the present study, increased numbers of vibrios and spirochetes other than *T. hyodysenteriae* have been observed in swine dysentery (22, 23); however, the presence of large numbers of a particular organism in diarrheal disease does not necessarily reflect its pathogenicity (15, 25). In addition, the controversy regarding the pathogenic role of *T. trichiura* in man may be explained by an interaction between the nematode and the gut flora; although Jung and Beaver (24) observed that *Entamoeba histolytica* was frequently present in association with human trichuriasis, *T. trichiura* has also been reported to be pathogenic in the absence of *E. histolytica* (10). In contrast, Hartz (20) found that no clinical signs and minimal damage to the colonic mucosa occur in *T. trichiura* infection. These results could be explained by the presence or absence of microbial components in the large intestine at the time of parasitic infection.

The microbial flora of the large intestine of animals includes fusiform bacteria, vibrios, and spirochetes (12, 13, 25, 29). Although these bacteria are not generally regarded as being pathogenic, they may enhance a clinical syndrome if they proliferate to reach numbers greater than are normally present. This may occur only if the ecological niche is disturbed, e.g., by excessive production of mucus as a result of damage to the large intestine. However, administration of the bacteria alone may not reproduce the disease in normal animals unless the environment of the gut is modified to provide suitable conditions for bacterial proliferation.

**ACKNOWLEDGMENTS**

We are indebted to C. Lane-Petter, J. Bell, N. Hammet, M. Burrows, and C. Jay for their expert assistance, and to B. Kitchenham for statistical analyses. We thank M. Hoare, C. Davies, and M. Dennis for providing the gnotobiotic piglets, P. Bland for the electron micrographs, and I. Jebbett for preparing the photographs.

**LITERATURE CITED**

4. Batte, E. G., and D. J. Moncol. 1972. Whipworms and