Transmissible Gastroenteritis in Feeder Swine: Clinical, Immunofluorescence and Histopathological Obervations

M. Morin, L. G. Morehouse, R. F. Solorzano and L. D. Olson*

ABSTRACT

Eight feeder swine (four to six months of age) were inoculated orally with 200,000 to 500,000 pig infectious doses (PID) of the Purdue strain of transmissible gastroenteritis (TGE) virus. Biopsies obtained from their small intestines were examined histopathologically and by fluorescent antibody tissue section technique at intervals that included 24, 48, 72 and 96 hours postexposure, and similar examinations were carried out at necropsy 168 hours postexposure. Evidence of virus infection was demonstrated in all segments of the small intestine except the upper duodenum and the viral antigen was found only in the cytoplasm of the absorptive cells covering the villi. Although six of the eight pigs failed to show clinical signs of TGE, typical microscopic lesions of villous atrophy with replacement of columnar absorptive cells by cuboidal cells were observed in seven pigs, and TGE virus antigen was demonstrated in the intestinal cells of four of eight pigs during the first week postexposure. The infection was usually mild to moderate and focal in the pigs without clinical signs of the disease and more severe and extensive in the pigs with clinical signs of the disease variable in severity. It was concluded that TGE virus probably replicated in all feeder swine exposed, and that the presence or absence of clinical signs of TGE in these pigs was related to the severity and extent of the villous atrophy and columnar cell replacement induced in their small intestines.

RÉSUMÉ

On a administré à huit porcs à l'engraissement (âgés de quatre à six mois), par la voie buccale, de 200,000 à 500,000 doses infectieuses porcines (DIP) de la souche "Purdue" du virus de la gastro-entérite transmissible (GET). On préleva des biopsies de leur intestin grêle et on les examina au moyen de l'histopathologie et de l'immunofluorescence, à intervalles de 24, 48, 72 et 96 heures après l'infection; on procéda à des examens similaires au moment de la nécropsie, c'est-à-dire 168 heures après l'infection. On démontra l'évidence de l'infection dans tous les segments de l'intestin grêle, sauf dans le duodénum proximal, et on ne décela l'antigène viral que dans le cytoplasme des cellules absorbantes bordant les villosités. En dépit du fait que six des huit porcs ne manifestèrent aucun signe clinique de GET, on observa quand même des lésions microscopiques caractéristiques d'atrophie des villosités, avec remplacement des cellules cylindriques absorbantes par ces cellules cubiques, chez sept sujets; on décela aussi l'antigène viral de la GET dans les cellules de l'intestin de quatre des huit porcs, au cours de la première semaine après l'infection. En général, cette infection se traduisit par des foyers où les lésions étaient bénignes ou modérées, chez les porcs qui ne manifestèrent pas de signes cliniques de la maladie; elle s'avéra cependant plus grave et plus

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diffuse chez ceux qui présentèrent des signes cliniques plus ou moins prononcés. On conclut que le virus de la GET se multiplia probablement chez tous les porcs à l'engraissement exposés à la maladie et que la présence ou l'absence de signes cliniques de GET chez ces sujets fut reliée à la gravité et à l'étendue de l'atrophie des villosités et du remplacement des cellules cylindriques provoquées dans leur intestin grêle.

INTRODUCTION

Transmissible gastroenteritis (TGE) is an enteric disease of swine caused by a virus and characterized by vomition, profuse diarrhea, dehydration, and a high mortality in piglets under two weeks of age (5). The TGE virus replicates through all segments of the small intestine of baby pigs with the highest titer in the jejunum (11). Using immunofluorescence and the electron microscope to study the small intestinal mucosa of baby pigs infected with TGE virus, it appeared that the virus replication occurred only in the cytoplasm of the absorptive cells on the villi, but not in the undifferentiated cells of the crypts of Leiberkühn (16, 17). The infection caused a rapid destruction of the villous epithelium which led to a villous atrophy (10, 12, 13, 18).

Clinical signs of TGE are frequently observed in feeder swine on the same farm having problems with the disease in baby pigs. In epizootiological studies, it appeared that the disease often started in feeder pigs and then spread to baby pigs and their dams on the same farm (3, 6, 7). The disease was also observed to affect entire herds of feeder swine when baby pigs were not present on the farm (5). In older pigs diarrhea can be profuse and severe, but this is usually transitory and the mortality is low. These pigs have been suspected to be a reservoir of TGE infection and to play an important role in the virus survival during summer (8). The disease has been reproduced experimentally in five to six month old swine (14).

The study reported herein was undertaken to make clinical observations and immunofluorescence and histopathological studies of the intestinal tracts of individual feeder pigs following oral exposure to the TGE virus.

MATERIALS AND METHODS

VIRUS

Two inocula were prepared with the Purdue strain of TGE virus.[1] The first inoculum was a homogenate of the small intestine and its content from a five day old pig 48 hours following oral exposure to the virus. This 20% homogenate in phosphate buffered saline (PBS) was centrifuged at 1,000 X g for 30 minutes and the supernatant fluid was frozen in screw-capped vials at -70°C. The second inoculum was a pool of undiluted intestinal content collected from two baby pigs 48 hours after oral exposure to the virus. These two inocula contained approximately 10^5 pig infectious doses (PID) per mililiter as determined by titration in baby pigs.

PIGS

Nine pigs (eight infected, one control) approximately four to six months old were used in the study. Eight pigs were from a secondary SPF herd maintained by the Department of Veterinary Pathology at the University of Missouri, and one pig (No. 7) was purchased from a private farm with no history of TGE. Two pigs received approximately 200,000 PID of the first inoculum orally, and six pigs received approximately 500,000 PID of the second inoculum by the same route (Table I). The study was conducted with one pig at a time. Clinical signs of TGE were recorded. Sequential biopsies were taken from the jejunum of these pigs through a laparotomy incision in the left flank. The schedule of the biopsies performed on each pig is shown (Table I). Surgical anesthesia was obtained in the following manner: a tranquilizer, (phencyclidine hydrochloride)[2] was injected intramuscularly at a dosage of 0.8 mg per kg of body weight. The pig was immobilized within five to eight minutes after injection. Surgical anesthesia was then induced with ether.[3] After the laparotomy incision was

[1]Obtained from Dr. E. O. Haelterman, Department of Veterinary Microbiology, Pathology and Public Health, School of Veterinary Science and Medicine, Purdue University, Lafayette, Indiana.

[2]Sernylan(R), Bio-Ceutic Laboratories, Inc., St. Joseph, Missouri.

[3]Ether Squibb, E. R. Squibb and Sons, New York, New York.

TABLE I. Clinical Signs of TGE and Results of Immunofluorescence and Histopathological Examinations on Biopsy and Postmortem Specimens

Pig No.	Inoculum	Clinical[a] Signs		Immunofluorescence (FA) and Histological Lesions (HL)											
				Biopsy Schedule (Hours Post-Exposure)					Necropsy						
				24	48	72	96	168	Hours Post-exposure	U. Duo.	L. Duo.	U. Jej.	M. Jej.	L. Jej.	Ileum.
1[d]	None	−	FA[b]	—	—		−		168	−	−	−	−	−	−
			HL[c]	−	−		−			−	−	−	−	−	−
2	2 x 10^5 PID	−	FA	—	—		+		168	−	+	+	+	+ +	+
			HL	++	−		++			−	−	+	−	+ +	+
3	”	−	FA	−	−		−		168	−	−	−	−	−	−
			HL	+	+		+			−	+	−	−	+	+
4	1 x 10^5 PID	−	FA	−	−		−		168	−	−	−	−	−	−
			HL	−	+		+			−	−	−	−	−	−
5	”	+	FA	−	+++		++		168	−	−	−	−	−	−
			HL	−	++		+++			−	−	*	+++	+++	+
6	”	−	FA			+		−	360	−	−	−	−	−	−
			HL			−		−		−	−	−	−	−	−
7	”	−	FA	−	−				168	−	−	+	+	−	−
			HL	−	++					*	*	−	+	+	−
8	”	−	FA	−					72	−	−	−	−	−	−
			HL	++						−	−	++	−	+	−
9	”	++	FA		−		−		168	−	−	−	−	−	−
			HL		+++		+			−	−	++	+++	+++	+

[a]Clinical signs of TGE
− = no signs of enteric disorders
+ = soft stool
++ = diarrhea

[b]Demonstration of TGE virus by immunofluorescence
− = negative
+ = occasional absorptive cells positive
++ = larger number of positive cells, but still focal
+++ = several villi with a large number of their adsorptive cells positive

[c] Microscopic lesions of TGE
− = negative
+ = lesions very focal. Most of the villi in the sections were normal
++ = more extensive lesions, but a number of villi were still normal
+++ = extensive and severe lesions. Most of the villi were involved

[d] Uninfected control

* Autolytic changes making interpretation difficult

made, a loop of jejunum approximately 4.0 inches long was exposed and a biopsy was obtained with curved scissors midway between the mesenteric and the antimesenteric borders of the gut. The surgical procedure was the same as one described for simple enterotomy in the dog (1). The gut sample obtained was divided immediately for the fluorescent antibody tissue section technique (FAT) and histopathological examinations.

At the time of necropsy (Table I), samples were obtained from the upper duodenum, lower duodenum, upper jejunum, midjejunum, lower jejunum, ileum, tonsil, pancreas, spleen and mesenteric lymph nodes for the same purpose as listed for the biopsies. The gut samples were obtained from the wall of the small intestine in a similar manner to that described for the biopsy. The villi were immediately placed in contact with formalin for rapid fixation.

Specimens for the FAT were placed in screw-capped vials and rapidly frozen in a mixture of dry ice and alcohol and stored at -70°C. At the time of sectioning, they were embedded with OCT embedding medium[4] on the cryostat specimen holder, and the gut samples were oriented to obtain longitudinal sections of the villi. Sections were cut 8.0 μ thick in a cryostat at –28°C. The slides obtained were allowed to dry for 15 minutes at 37°C and were then fixed in acetone at -15°C for 15 minutes. After fixation, they were dried for ten minutes at 37°C and then stored in a sealed box at -15°C until ready for immunofluorescent staining.

Specimens collected for histopathology were fixed in 10% neutral buffered formalin and processed for paraffin tissue sections according to conventional methods. Sections were cut 6.0 to 8.0 μ thick and stained with hematoxylin and eosin.

Fluorescent Antibody

The conjugate was prepared from the serum of a 12 week old pig hyperimmunized with the Purdue strain of TGE virus. The pig was first exposed orally at six weeks of age with 2.0 ml of a 20% intestinal homogenate containing the virus and developed a severe diarrhea. The inoculum for two subsequent doses of virus was prepared in the following manner: 70.0 ml of the virus-containing intestinal homogenate was centrifuged at 14,500 X g for one hour at 5°C. The supernatant fluid obtained (50.0 ml) was passed through a 0.22 mμ filter and then centrifuged at 105,500 X g for five hours at 5°C. The supernatant fluid was discarded and the pellets obtained were reconstituted with 13.0 ml of a sterile PBS solution, at pH 7.3. Twenty-one days after the initial oral exposure, 2.0 ml of this inoculum were injected intramuscularly. A second injection was given subcutaneously fourteen days later with 2.0 ml of the inoculum mixed with 2 ml of Freund's adjuvant. Fourteen days later, the pig was exsanguinated and its serum collected. The antibody titer was estimated by using a serum neutralization test performed in cell culture using a cytopathogenic effect of the Miller strain of TGE virus[5] in a swine testis cell line[6] as the indicator system. The serum had the ability to neutralize 1,000

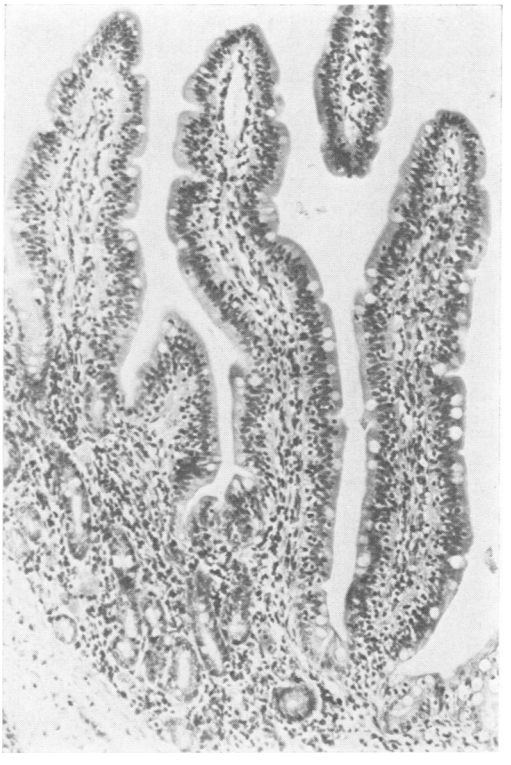

Fig. 1. Normal jejunal mucosa of a four month old pig. Notice the elongated villi covered by columnar epithelial cells. H & E X450.

[4]Tissue-Tek, Ames Co., Elkhart, Indiana.

[5]Obtained from Dr. E. H. Bohl, Department of Veterinary Science, The Ohio Agricultural Research and Development Center, Wooster, Ohio.

[6]Obtained from Dr. A. W. McClurkin, National Animal Disease Laboratory, Ames, Iowa.

tissue culture infectious doses (TCID) of cytopathogenic virus at a dilution of 1:1600. The methods used for the extraction of the gamma globulin fraction and the labelling of this fraction with fluorescein isothiocyanate[7] were those used to prepare conjugate for hog cholera (20). The staining procedures used were those routinely employed for the detection of hog cholera virus (2) by the FAT. Specificity of the fluorescence was tested in the following manner: frozen sections from the small intestines of uninfected pigs, when stained with the fluorescent antibody preparation, failed to show fluorescence similar to that seen in the sections from pigs exposed to TGE virus. Specificity was also demonstrated by using one-step and two-step inhibition tests; the anti-TGE antiserum used as inhibitor in these tests was that from which the conjugate was prepared. Finally, gut sections from TGE-infected pigs were stained with anti-hog cholera, anti-distemper, and anti-bovine virus diarrhea virus conjugates; fluorescence similar to that produced by the anti-TGE conjugate was not observed.

[7]B.B.L., Division of Bioquest, Cockeysville, Maryland.

RESULTS

Clinical Signs

All pigs exposed to the TGE virus were depressed ten to 24 hours following exposure; however, only pig No. 5 had soft stools 48 hours after exposure and pig No. 9 had diarrhea at 24 hours, which persisted for three days (Table I).

Gross Lesions

During surgery, grossly visible lesions in the gut were seen only in pig No. 5, 48 hours postexposure. The lesion in the segment of small intestine, which was exam-

TABLE II. Summary Comparing Results of the Immunofluorescence and Histopathological Studies

Pigs	1[c]	2	3	4	5	6	7	8	9
Immunofluorescence[a]	−	+	−	−	+	+	+	−	−
Histological lesions[b]	−	+	+	+	+	−	+	+	+

[a]Demonstration of TGE virus by immunofluorescence
− = Negative
+ = Viral antigen was demonstrated
[b]Microscopic lesions of TGE
− = Microscopic lesions were not demonstrated
+ = Microscopic lesions were demonstrated
[c]Pig No. 1 was an uninfected control

TABLE III. Correlation of Clinical Signs of TGE with Severity of Small Intestinal Infection as Estimated from Results of the Immunofluorescence and/or Histopathological Studies

Pigs	1[c]	2	3	4	5	6	7	8	9
Clinical signs[a]	−	−	−	−	+	−	−	−	++
Intestinal Infection[b]	−	++	+	+	+++	+	++	++	+++

[a]Clinical signs of TGE
− = No signs of enteric disorders
+ = Soft stool
++ = Diarrhea
[b]Small intestinal infection by TGE virus
− = No evidence of intestinal infection
+ = Infection mild, very focal in nature
++ = Infection moderate but still focal
+++ = More severe and extensive infection
[c]Pig No. 1 was an uninfected control

ined, consisted of a thin appearance of the wall and distention of this segment by yellow fluid. On postmortem examination, similar lesions were observed in some segments of the small intestine of pig No. 9.

Immunofluorescence and Histopathological Studies

TGE antigen was detected by the FAT in four of eight pigs that had been exposed to the virus and microscopic lesions usually associated with the disease were observed in seven of the eight pigs (Tables I and II). Neither lesions nor positive fluorescence were seen in the noninfected control pig (Figs. 1 and 2).

The TGE viral antigen was detected only in the cytoplasm of the absorptive epithelial cells covering the villi of the small intestine and was not seen in the epithelial cells lining the crypts of Lieberkühn. Specific fluorescence was usually bright, apple green in color, granular and often more intense in the apical cytoplasm of the cell (Figs. 4 and 9). This was in contrast to the dull green color of noninfected epithelial cells (Fig. 2).

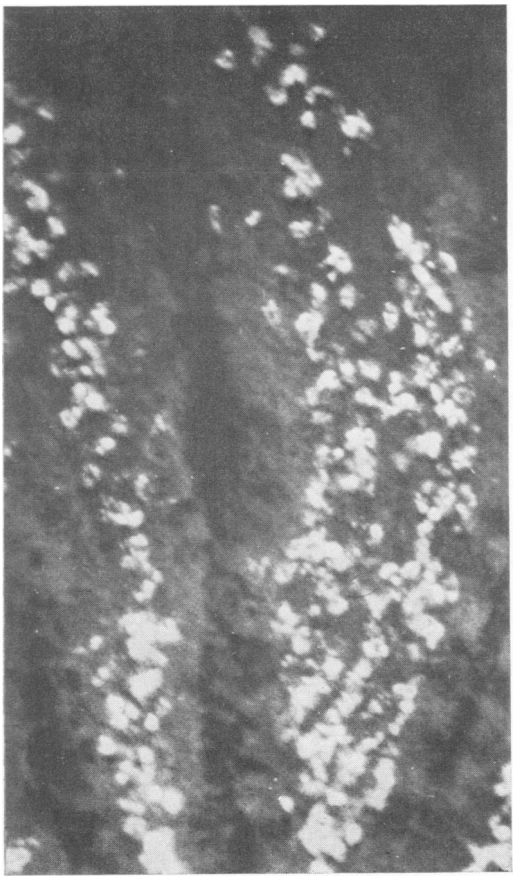

Fig. 2. Normal jejunal villi stained with fluorescent antibody. Notice the bright nonspecific fluorescence produced by eosinophils infiltrated in the villous core. X250.

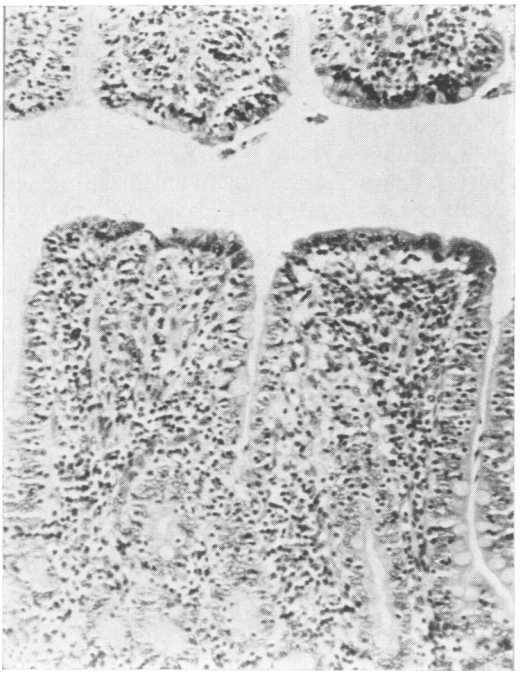

Fig. 3. Biopsy obtained from the jejunum of pig No. 5, 48 hours after exposure to TGE virus. Notice villi shorter than normal and blunted. Cells covering their upper part are undergoing degeneration. H & E X420.

The following microscopic lesions were observed in the biopsy and postmortem samples obtained from the small intestine of the infected pigs: 1) villous atrophy and blunting variable in severity (Figs. 5 and 8); 2) replacement of the absorptive columnar epithelial cells by flat to cuboidal immature epithelial cells (Figs. 5, 6 and 8); and 3) thickening of the intercryptal lamina propria, and elongation of the crypts of Lieberkühn (Figs. 5 and 8). Edema occasionally could explain the thickening of the lamina propria (Fig. 5), but in most instances, this change was not present.

Lesions were observed in the small intestine of all the pigs exposed to the virus (Table III). In pigs No. 2, 3, 4, 6, 7 and 8, it was usually mild to moderate and focal in nature. Lesions in pig No. 2 were representative of the group (Table I). In the biopsy obtained at 24 hours postexposure (PE), no viral antigen was detected by the FAT but a number of foci of villous atrophy

and replacement of absorptive columnar cells by cuboidal cells were present. In the biopsy obtained 96 hours PE, TGE antigen was detected in a few of the absorptive epithelial cells (Fig. 9). Lesions of TGE were moderate in severity and focal. At the time of necropsy, one week after exposure, viral antigen was still present in all segments of the small intestine with exception of the upper duodenum. Usually, only a few cells were involved, but in the section obtained from the lower jejunum, almost all the epithelial cells covering the villi had viral antigen in their cytoplasm. Microscopic lesions were also present in some segments, but they were focal, and a number of normal villi were present in the sections (Fig. 8). TGE antigen was not demonstrated by the FAT in pigs No. 3, 4, and 8 but typical microscopic lesions of villous atrophy were detected. It should be pointed out here that TGE antigen was demonstrated by the FAT

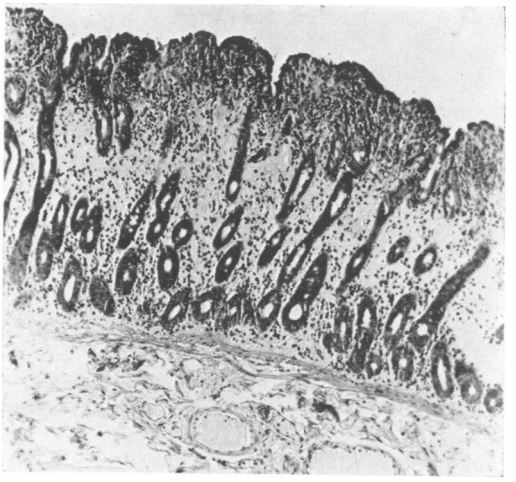

Fig. 5. Biopsy obtained from pig No. 5, 96 hours post-exposure. Notice the severe villous atrophy, thickening of the lamina propria and elongation of the crypts. H & E X155.

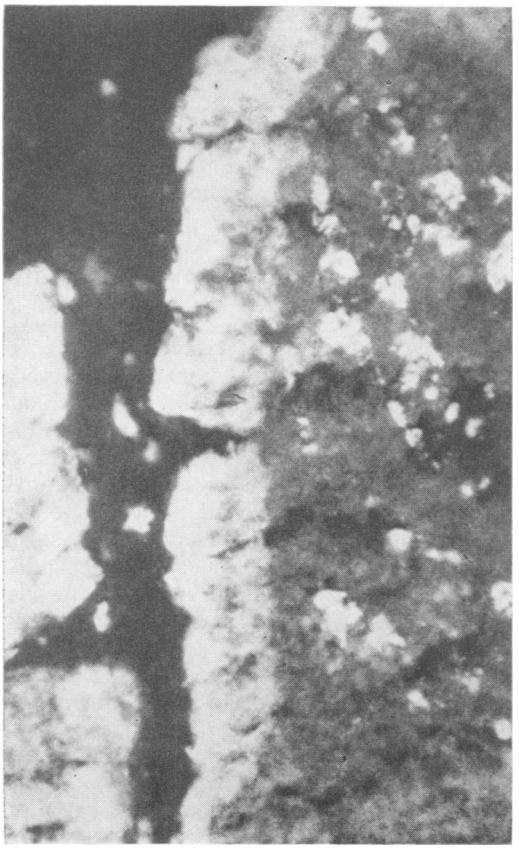

Fig. 4. Fluorescent antibody stained section from the biopsy in Figure 3. All the epithelial cells covering the villi have a large amount of viral antigen in their cytoplasm. X550.

in the gut of two pigs (Nos. 2 and 7), that had never shown clinical signs of enteric disorder, one week after their exposure to the virus. Signs of severe and extensive intestinal infection were observed in pigs No. 5 and 9. In the biopsy obtained from pig No. 5 at 48 hours PE, almost all the absorptive cells covering the villi had viral antigen in their cytoplasm (Fig. 4). Several of these villi were blunted, moderately shorter than normal, and several of their absorptive cells were undergoing degeneration and desquamation (Fig. 3). In the biopsy obtained at 96 hours PE, severe and extensive lesions of villous atrophy and replacement of columnar cells by cuboidal immature cells were present (Figs. 5 and 6). Some of the immature cells covering the villi had viral antigen in their cytoplasm, but the number of positive cells had decreased significantly (Fig. 7). At the time of necropsy, three days later, severe lesions of TGE were still present in the jejunum but there were signs of villous regeneration. In several areas, villous atrophy was not as severe and several villi were covered by epithelial cells which were almost columnar. Viral antigen was not detected by the FAT in those cells. Similar histopathological observations were made in pig No. 9 but viral antigen was not detected by the FAT.

Viral antigen was not detected in the tonsil, mesenteric lymph nodes, spleen or pancreas of any of the pigs.

DISCUSSION

From the results of this study, it is suggested that the site of replication of the TGE virus in feeder swine is the same as that observed in baby pigs (11, 16). Evidence of virus infection was seen in all segments of the small intestine except the upper duodenum and the viral antigen was found only in the cytoplasm of the absorptive cells covering the villi. The crypt cells were not involved. The constant presence of eosinophils in the villous core and the intercryptal lamina propria resulted in varying degrees of nonspecific fluorescence; however, they were easily identified because of their location and their characteristic appearance in the presence of incandescent light. The microscopic lesions observed in this study were very similar to those that have been described for TGE infection in baby pigs (12, 13, 18) and feeder swine (14). Pensaert *et al* (16), were unable to demonstrate TGE viral activity by immunofluorescence either in intact baby pigs or in isolated intestinal loops seven days PE. According to those workers, villi in the intestines of the baby pigs that had survived the disease had almost reached their original length after seven days. In the study reported herein, severe lesions of villous atrophy with evidence of a beginning of villous regeneration were observed in two of the pigs killed seven days PE. Viral antigen was also detected by the FAT in the jejunal cells of two of the pigs killed seven days PE. Thus, there appears to be a distinct chronological difference in the development of TGE lesions in the different age groups of pigs.

In this study, all the pigs exposed to TGE virus had evidence of the infection in their small intestines despite the fact that only two of them had clinical signs of enteric disorders. The infection was mild to moderate and focal in the pigs without clinical signs of TGE and more severe and extensive in the pigs which had a diarrhea or soft stool. From these observations, it is suggested that the presence or absence of clinical signs

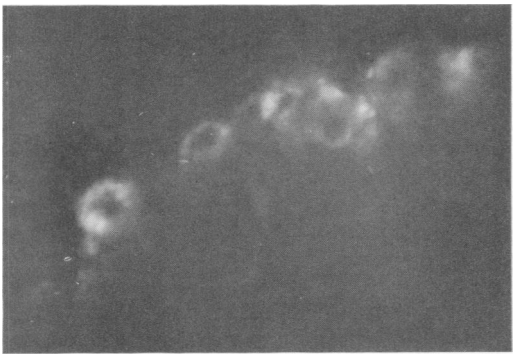

Fig. 7. Fluorescent antibody stained section from the biopsy in Figures 5 and 6. Notice the presence of few cells with viral antigen in their cytoplasm. X730.

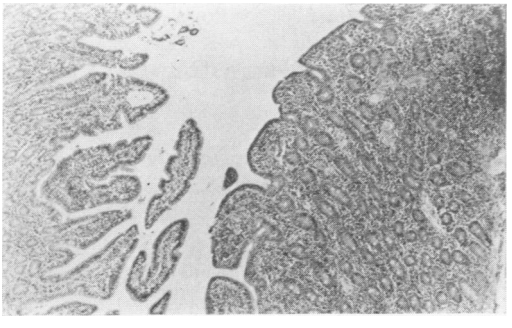

Fig. 8. Section from the jejunum of pig No. 2, one week after exposure to TGE virus. A focal area of villous atrophy can be seen on the right. Notice the presence of normal villi on the left. H & E X100.

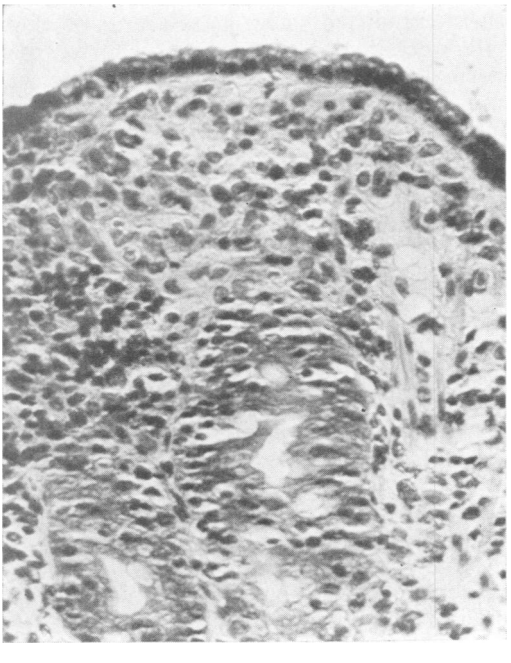

Fig. 6. Same biopsy as that shown in Figure 5. Notice the flat to cuboidal cells lining the atrophic villi. H & E X900.

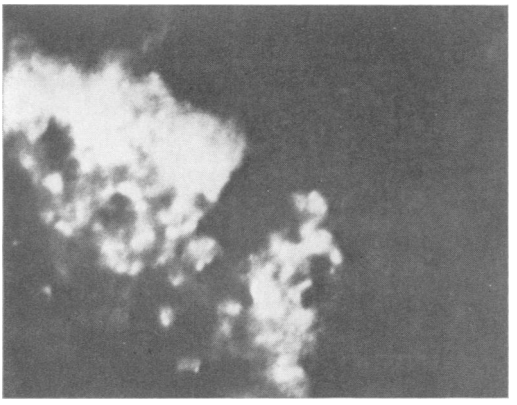

Fig. 9. Fluorescent antibody stained section from the biopsy obtained from pig No. 2, 96 hours postexposure. Notice the presence of a few cells with specific fluorescence in their cytoplasm. X900.

of TGE in feeder swine exposed to the virus is related to the severity and extent of the villous atrophy and cell replacement induced by the virus. In baby pigs infected with TGE virus, the immature cells covering the atrophic villi have an abnormal microvillous border (12, 13, 19) and because of this, these cells are probably poor in several important hydrolytic enzymes (3, 13, 19) that are normally present in this region. These changes, combined with the villous atrophy, cause a maldigestion and malabsorption of nutrients because of a loss of the digestive-absorptive surface of the small intestine (4, 9, 10, 13, 19). In feeder swine with focal lesions, the loss of digestive-absorptive surface is probably not severe enough to induce visible signs of diarrhea or soft stool. However, in those instances where more extensive lesions develop, the surface loss is too severe and this leads to visible clinical signs of enteric disorders such as soft stool or diarrhea variable in severity.

In pigs No. 3, 4, and 8, the lesions were suggestive of a less severe infection. Failure to demonstrate viral antigen in those pigs may have been simply a problem of sampling. Pig No. 9 was already in the atrophic stage of TGE when the first biopsy was obtained at 48 hours PE, and it has been noted in baby pigs (15) and in this study that the amount of viral antigen detectable by the FAT decreases significantly in this stage. It should be mentioned, however, that the TGE antigen was demonstrated in one pig (No. 6) which had shown neither lesions of TGE nor clinical signs of enteric disorders. This would suggest that a combination of immunofluorescence and histopathological examinations on specimens obtained from the jejunum may be the best approach for the confirmation of a diagnosis of TGE in feeder swine.

It appeared that TGE virus probably replicated in all susceptible feeder swine that were exposed. Apparently, some of these pigs had only a focal, mild to moderate infection and, likewise, suffered no apparent diarrhea or soft stool. Other pigs of the same age exposed to a similar amount of virus, had a more severe and extensive small intestinal involvement and displayed clinical signs of enteric disorders variable in severity. In this study, feeder swine were singly exposed to the virus and this may have accounted for the low incidence of severe infections. If several feeder swine in close contact are exposed to TGE virus and some of them (e.g. pigs No. 5 and 9) suffer a more severe intestinal infection, they probably will shed larger amounts of virus in their feces. This would contribute to an increase in the amount of virus to which the other pigs are exposed which might raise the incidence of severe infections and signs of enteric disorders.

Some authors (3, 8) feel that feeder swine may play an important role in the epizootiology of TGE. Results of this study suggesting that some feeder swine can support a focal growth of TGE virus in their small intestine without clinical signs of the disease, for at least one week after their exposure, stresses the importance for further studies in this area. Experimental and field studies are presently underway in our laboratory with the principal goal of further defining the role of feeder swine as a reservoir of TGE infection for baby pigs.

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