Experimental Transmission of Intestinal Coccidiosis to Piglets: Clinical, Parasitological and Pathological Findings

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ABSTRACT

Twenty-eight piglets coming from a "specific pathogen free" herd were inoculated at three days of age with 50 000 or 100 000 sporulated oocysts of *Isospora suis*. Fecal samples were examined for oocyst shedding daily and several clinical parameters were recorded. Ten piglets were used as normal controls. Groups of piglets were euthanized from three days to 12 days postinoculation and routine necropsies were performed. Bacteriological, virological, parasitological and histopathological examinations were made on the intestinal tracts.

The incubation period was four to five days. Clinical signs and microscopic intestinal lesions observed in the experimentally infected animals were similar to those reported in spontaneous cases of porcine neonatal coccidiosis. Lesions of villous atrophy in the small intestine seemed to result from the destruction of villous epithelial cells mainly during the peak of asexual reproduction which occurred around four to five days postinoculation. Intracellular coccidial organisms were difficult to find during the late atrophic and villous regrowth stages of the intestinal lesions.

The prepatent period varied from four to seven days and the most common was five days. Eighty percent of the piglets kept alive more than four days postinoculation have shed oocysts. Piglets dosed with old sporulated oocysts (ten months old) shed many more oocysts than those infected with a fresh inoculum (less than two months old). The patent period was not determined precisely with the design of the experiment but some of the infected piglets shed oocysts for at least five days.

Key words: *Isospora suis*, piglets, intestinal coccidiosis, villous atrophy.

RÉSUMÉ

Cette étude portait sur 28 porcelets provenant d'un troupeau exempt d'agents pathogènes spécifiques que les auteurs inoculèrent dès l'âge de trois jours avec des doses de 50 000 ou 100 000 oocystes sporulés d'*Isospora suis*. Des échantillons de selles étaient examinés quotidiennement et plusieurs paramètres cliniques servaient à évaluer l'état de santé des porcelets infectés. Dix porcelets servirent de contrôles sains. Des nécropsies furent effectuées sur les porcelets euthanasiés entre le troisième et le douzième jour post-inoculation. Des examens bactériologiques, virologiques, parasitologiques et histopathologiques furent effectués à partir des intestins des porcelets nécropsiés.

Les signes cliniques et les lésions intestinales observées chez les porcelets infectés se sont avérés semblables à ceux déjà décrits dans les cas naturels de coccidiose néonatale porcine. Des lésions d'atrophie villositaire dans le grêle semblent avoir été provoquées par la destruction des entérocytes matures qui s'est produite surtout durant le pic de la reproduction asexuée qui survint à quatre ou cinq jours post-inoculation. La détection de formes coccidiennes intracellulaires s'est avérée difficile lorsque les lésions intestinales se retrouvaient à la fin de la phase atrophiante ou au début de la phase de régénérescence des villosités affectées.

La période de prépatence a varié de quatre à sept jours, mais la plus commune fut de cinq jours. Quatre-vingt pour cent des porcelets gardés vivants plus de quatre jours post-inoculation ont éliminé des oocystes dans leurs selles. Les porcelets infectés avec des oocystes âgés (sporulés depuis

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dix mois) ont éliminé un nombre beaucoup plus élevé d'oocystes que ceux infectés avec un inoculum frais (âgé de moins de deux mois). La durée du passage des oocystes dans les selles n'a pu être déterminée avec certitude mais certains des porcelets en passèrent pendant au moins cinq jours.

Mots clés: *Isospora suis*, porcelets, coccidiose intestinale, atrophie villositaire.

INTRODUCTION

The coccidium *Isospora suis* has been incriminated as a cause of diarrhea in piglets by many diagnostic pathologists in recent years (1, 2, 3, 4, 5, 6, 7, 8, 9, 10). The disease is characterized by a yellowish, fetid diarrhea with a watery or greasy consistency, and by dehydration, rough hair coat and retarded growth mainly in piglets between five and 15 days of age with a peak occurrence between seven and ten days (7, 9). Morbidity rates are variable, but mortality rates are usually low. Microscopic lesions are confined to the jejunum and ileum and consist of a multifocal villous atrophy and blunting with focal ulceration to a severe fibrinonecrotic enteritis; coccidial organisms are present in variable numbers of epithelial cells covering normal or affected villi (3, 5, 6, 7, 9, 10).

Experimental reproduction of the disease has been reported recently (11, 12, 13). The main objective of the work reported here was to confirm *I. suis* as a determinant cause of neonatal diarrhea in piglets. A better understanding of the pathogenesis of the disease was another important goal.

MATERIALS AND METHODS

Inocula of *I. suis* used in this study were obtained from natural or experimental cases of porcine neonatal coccidiosis. Oocysts in colonic contents and feces were sporulated in 2.5% potassium dichromate at room temperature. The sporulated oocysts were then harvested via centrifugation at 1 000 × g for ten minutes, and the supernatant discarded. The sediment was diluted with a saturated sodium chloride solution and centrifuged at 1 500 × g for five minutes. The supernatant was recovered and mixed in a liter cylinder of tap water. Twelve hours later, the supernatant was removed by aspiration down to 100 mL of the bottom; the bottom liquid and sediment were recovered and centrifuged five minutes at 400 × g. The oocyst pellet was resuspended and diluted to obtain approximately 30 000 sporulated oocysts per mL of solution. The oocysts were counted with a hemacytometer.

Thirty-eight piglets were obtained from a specific pathogen free (SPF) herd with no history of neonatal diarrhea. Fecal samples from several sows of the herd examined for the presence of coccidia were negative. The piglets born naturally were allowed to suck their dam until two days of age and were then housed in individual isolation units similar to those described by Olson (14). They were fed a commercial sow's milk replacer (SPF-Lac, Borden Inc., Norfolk, Virginia) at a rate of 150 mL every eight hours.

Twenty-eight piglets were inoculated orally at three days of age: ten of these piglets received 50 000 young sporulated oocysts (sporulated oocysts less than two months old) while fifteen received 100 000 and three piglets were dosed with 100 000 old sporulated oocysts (sporulated oocysts ten months old). Ten piglets were used as normal controls. Fecal samples were examined for coccidia daily with the flotation and McMaster techniques; the appetite, stool consistency and general state of health were also recorded. Fecal samples were also examined daily for detection of enteropathogenic *E. coli* as already described (15).

Groups of piglets were euthanized from three to 12 days postinoculation (p.i.) and routine necropsies were performed. Specimens from the stomach, all levels of the small intestine and colon were fixed in 10% neutral buffered formalin and processed for parrafin tissue section according to conventional methods. Sections were stained with hematoxylin, phloxin and safran (HPS) and MacCallum-Goodpasture stains. For virological examinations, specimens obtained from the upper, middle and lower jejunum and ileum were rapidly frozen at -40°C. The fluorescent antibody tissue section technique was used for detection of transmissible gastroenteritis (TGE) virus (TGE virus conjugate supplied by the Institut Armand-Frappier, Laval des Rapides, Quebec) and rotavirus (Rotavirus conjugate supplied by Dr. E.H. Bohl, Ohio Agricultural Research and Developmental Center, Wooster Ohio) using methods already described (16). Direct electron microscopic examination of the jejunal and colonic contents was also performed after negative staining with 3% phosphotungstic acid (17) to detect the presence of viruses. Routine bacteriological examinations were also conducted on the intestinal tracts of these pigs; methods used to detect enteropathogenic *E. coli* (ETEC) have been reported recently (15). Routine parasitological examinations (flotation and McMaster techniques) were also performed on jejunal and colonic contents. Oocysts were identified according to the descriptions of Vetterling (18).

RESULTS

CLINICAL SIGNS

Twenty-three of the 28 inoculated piglets (82%) were anorexic and depressed four days p.i. Twenty-two of the inoculated piglets (78.6%) became diarrheic; diarrhea was noted for the first time four days p.i. in nine piglets (41% of the diarrheic piglets), five days p.i. in 12 (54.5%) and six days p.i. in one (4.5%).

Diarrheic feces were initially yellow colored, soft and milky, and were becoming watery and profuse within 24 hours. Anorexia,

depression, diarrhea or soft stools, dehydration and retarded growth were still present in piglets euthanized 11 and 12 days p.i. but mortality did not occur during the course of the experiment. Piglets used as normal controls remained normal.

OOCYST SHEDDING BY THE INFECTED PIGLETS

Sixteen of the 20 piglets (80%) kept alive more than four days p.i. have shed oocysts. Unsporulated oocysts were observed for the first time in feces or intestinal contents four days p.i. in three piglets (18.7% of the shedders), five days p.i. in nine (56.2%), six days p.i. in three (18.7%) and seven days p.i. in one (6.2%). The patent period could not be determined precisely with the design of the experiment, but some of the piglets shed oocysts for at least five days.

Oocyst counts varied from 50 to 558 000 oocyst per gram (OPG) of feces in the 16 shedders. Oocyst counts in seven piglets dosed with 50 000 young sporulated oocysts varied from 50 to 38 950 OPG of feces with a mean of 15 150 (total number of oocyst counts/number of inoculated piglets). Oocyst counts in ten piglets dosed with 100 000 young sporulated oocysts varied from 0 to 26 000 OPG of feces with a mean of 10 800. In the three piglets dosed with 100 000 old sporulated oocysts, oocysts counts varied from 1 000 to 558 000 OPG of feces with a mean of 320 000.

PATHOLOGICAL FINDINGS

Gross lesions were not observed in the five piglets euthanized three days p.i. Twenty-two of the 23 piglets killed between four and twelve days p.i. had liquid and yellow colored intestinal contents with no remarkable gross changes in their intestinal mucosa.

Microscopic lesions were not seen in the ten piglets used as normal controls (Fig. 1). In the infected piglets, lesions were restricted to the small intestines and were more frequent in the middle and lower jejunum and ileum. Two of the five piglets euthanized three days p.i. had a few foci of mild villous atrophy with ulcerations of the tip of a few villi; asexual stages of coccidia were rarely seen.

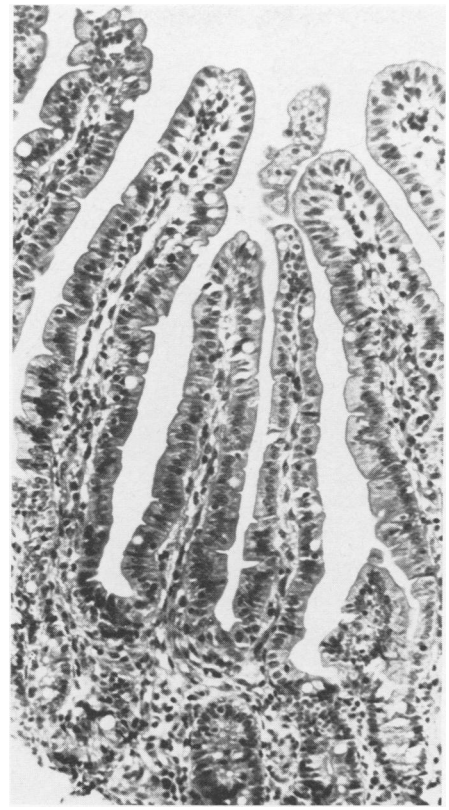

Fig. 1. Middle jejunum of a normal five day old piglet. HPS. X125.

Two of the three piglets euthanized four days p.i. had multifocal lesions of moderate to severe villous atrophy and blunting with focal ulcerations covered by a fibrinonecrotic exudate. The affected villi were covered by cuboidal or flat epithelial cells and the crypts of Lieberkühn were hyperplastic. Many asexual stages of coccidia (meronts, merozoites and schizonts) were present in epithelial cells lining normal or affected villi (Fig. 2). The crypts of Lieberkühn were not infected.

Fourteen piglets were euthanized between five and eight days p.i. These piglets all had intestinal lesions similar to those described in the previous group. Asexual forms of coccidia were still numerous in the piglets killed five days p.i. (Fig. 3) but they had decreased significantly in piglets killed later. Sexual forms of coccidia (micro-

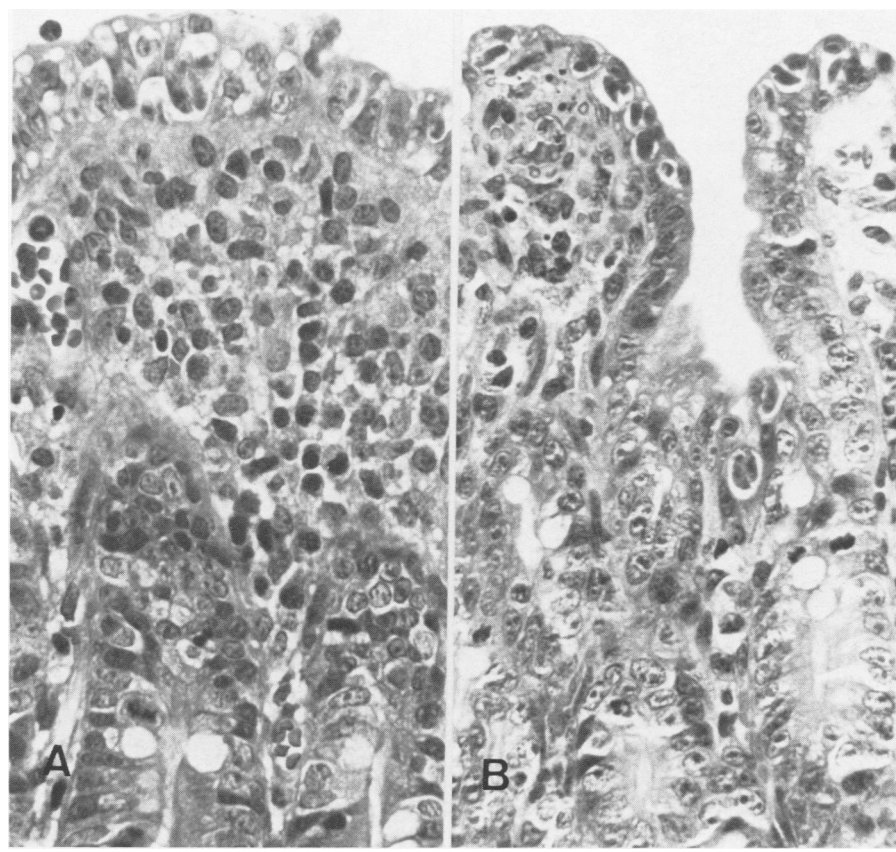

Fig. 2. Lower jejunum of two piglets euthanized four days p.i. Severe villous atrophy and large numbers of asexual stages of coccidia in villous epithelial cells. HPS. A. X300; B. X250.

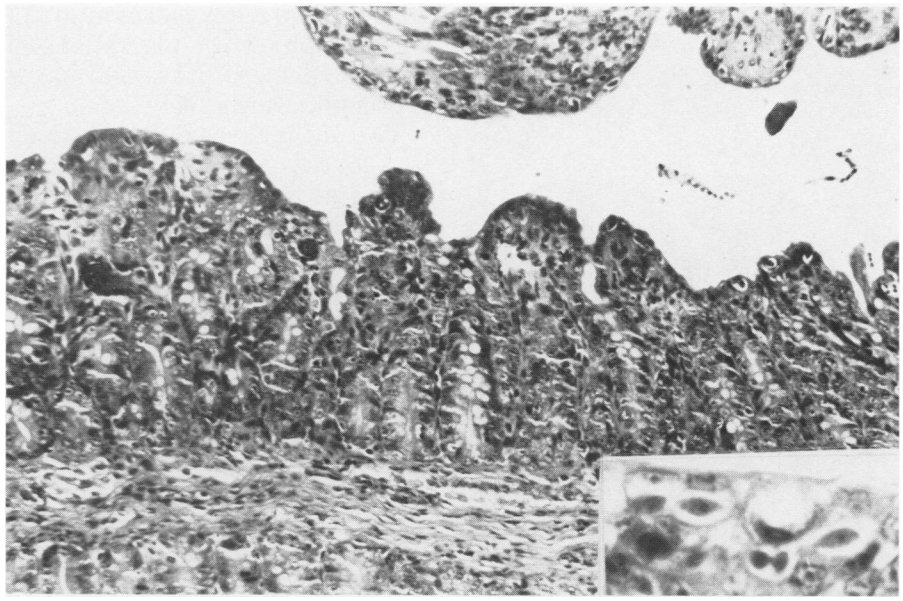

Fig. 3. Lower jejunum of a piglet euthanized five days p.i. Severe villous atrophy and replacement of mature columnar cells by cuboidal or flat epithelial cells. Crypts of Lieberkühn are hyperplastic and coccidial organisms are still numerous (asexual stages). HPS. X125. Insert: Higher magnification of the coccidial organisms. HPS. X300.

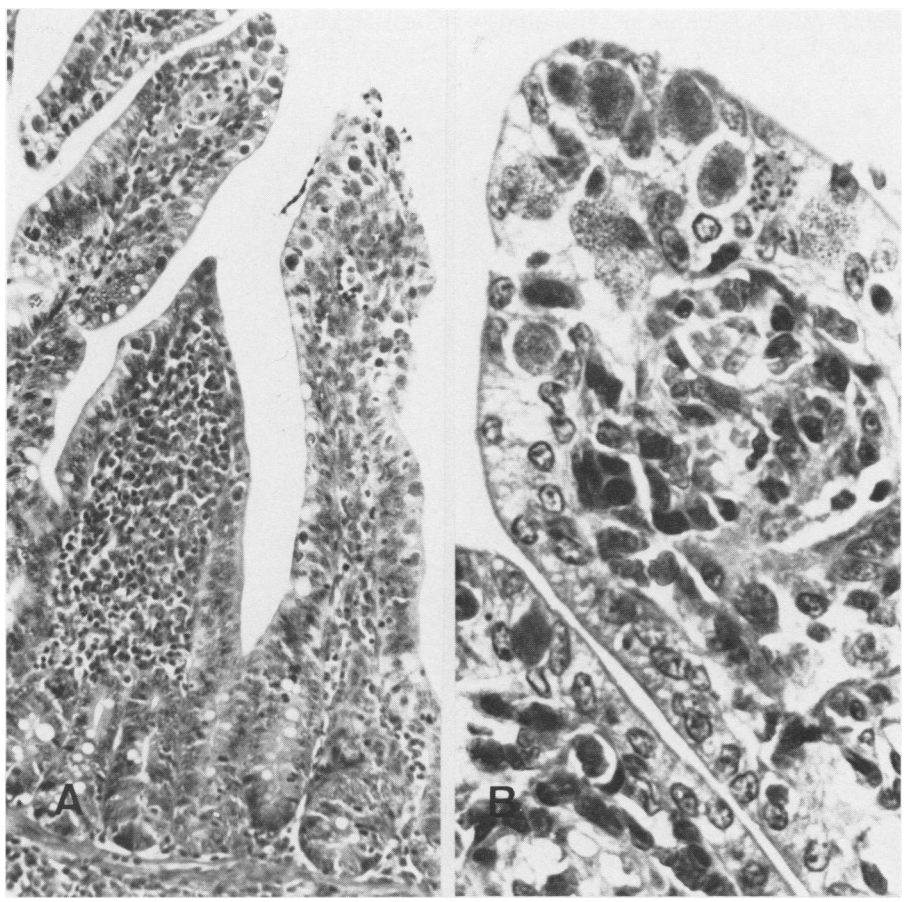

Fig. 4. Lower jejunum of a piglet euthanized five days p.i. A. Several coccidial forms, mainly sexual stages, are present in villous epithelial cells. HPS. X150. B. Macrogametocytes and one microgametocyte are present in epithelial cells lining the tip of the villous. HPS. X300.

gametocytes and macrogametocytes) were seen in significant numbers only in piglets killed five days p.i.; they were located mainly in the cytoplasm of columnar absorptive cells lining normal or atrophic villi (Fig. 4). In the piglets killed seven or eight days p.i., coccidial organisms were already difficult to find. Lesions of villous atrophy were still severe in those piglets (Fig. 5) but there were areas where the atrophy was not as severe with villi covered by low columnar epithelial cells.

Six piglets were euthanized between nine and 12 days p.i. These piglets still had multifocal areas of severe villous atrophy and blunting with focal ulcerations in their small intestines. Coccidial organisms were very difficult to find in those areas. These piglets also displayed several areas of villous regrowth. These villi were half or one-third of their normal length and were covered by low columnar or columnar epithelial cells (Fig. 6). The crypts of Lieberkühn were hyperplastic and coccidial organisms were almost impossible to find in those areas.

BACTERIOLOGICAL AND VIROLOGICAL FINDINGS

The bacteriological and virological examinations performed on the infected and control animals gave negative results.

DISCUSSION

Clinical signs and microscopic lesions observed in our experimentally infected animals were similar to those reported in spontaneous cases of porcine neonatal coccidiosis (1, 3, 4, 5, 7, 9); mortality and severe lesions of fibrinonecrotic enteritis were not observed in the present study. Stuart *et al* (19) have reported that the severity of the experimental disease is dose and age-related; several of the one to three day old piglets they had dosed with 200 000 to 400 000 oocysts, had severe clinical signs and a diphteric membrane within the jejunum and ileum, while the absence of gross lesions and a mild

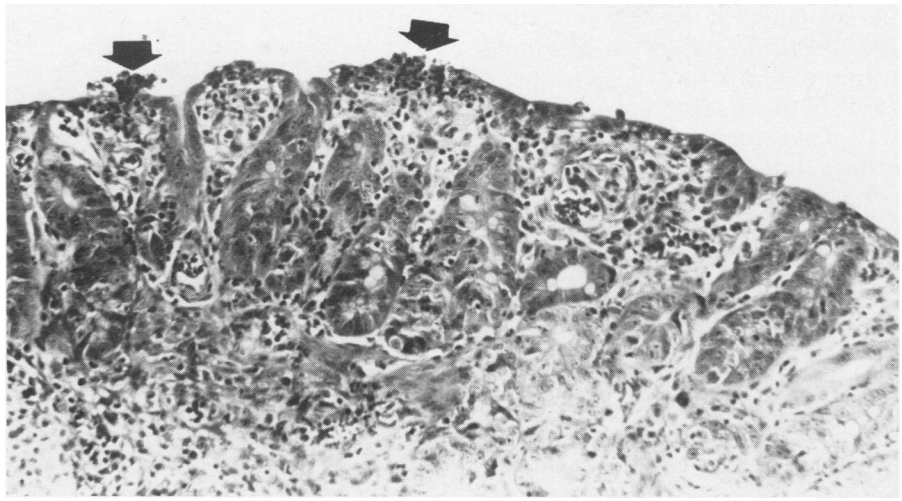

Fig. 5. Lower jejunum of a piglet euthanized seven days p.i. There is a severe villous atrophy with focal ulcerations of the mucosa (arrows) and the crypts are hyperplastic. Affected villi are covered by cuboidal cells and coccidia are absent. HPS. X150.

disease were noted in piglets infected at two to six weeks of age with 400 000 to 2 000 000 oocysts. Meyer (12) has reported also that lower oocyst doses cause mild disease, while higher doses (more than 150 000) cause severe dehydration and occasional mortality in gnotobiotic piglets. According to the observations made in the study reported herein, it is felt that 50 000 to 100 000 sporulated oocysts given to piglets under five days of age are sufficient to reproduce clinical signs and lesions very similar to the spontaneous disease. The use of 50 000 or 100 000 oocysts as infective doses caused a similar disease. The only difference noted resulted from dosing three piglets with an old inoculum and consisted in a longer incubation period.

Microscopic lesions were restricted to the small intestine and were characterized by a multifocal villous atrophy and blunting with focal ulcerations. These lesions seemed to result from the destruction of villous epithelial cells principally during the peak of asexual reproduction which seemed at its highest around four to five days p.i.; the gametogonic phase of the cycle also seemed to contribute to the lesions and its peak occurred around five days p.i. Similar findings have been reported recently (20, 21). Intracellular coccidial organisms were difficult to find in the late atrophic (cell replacement stage) and villous regrowth stages of the intestinal lesions; these lesions were almost impossible to differentiate from those induced by common viral enteropathogens infecting the villous epithelium such as TGE virus (22) and rotavirus (23). Diagnostic pathologists should be well aware of this fact before making a diagnosis of viral enteropathy based only on the presence of villous atrophy in diarrheic piglets older than five days of age. This marked reduction in the number of coccidial organisms visible in the mucosa when healing occurs has some similarities with viral enteropathies such as TGE in piglets where large amounts of virus are present only in the degenerative stage of the lesion (24).

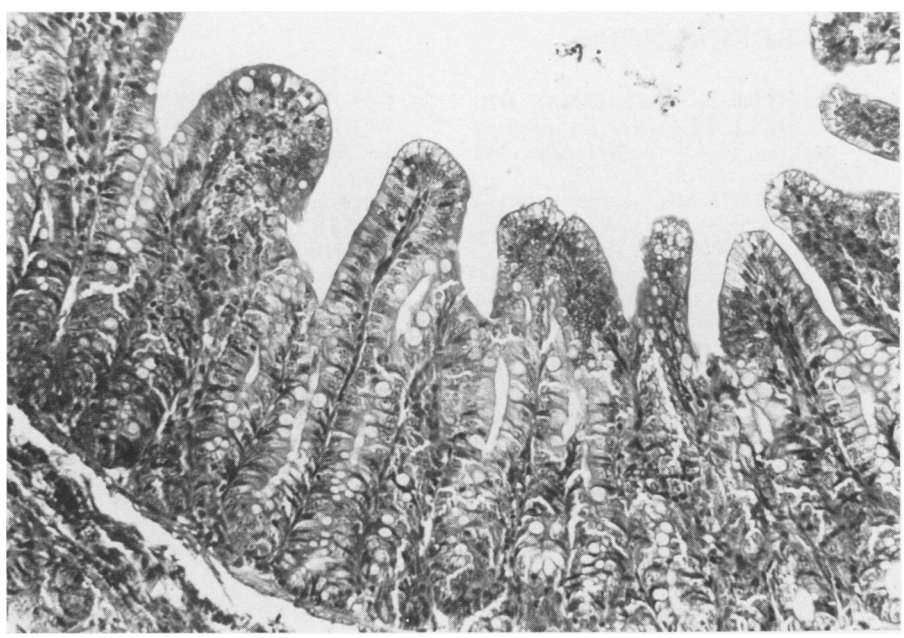

Fig. 6. Middle jejunum of a piglet euthanized ten days p.i. In this area, villi are one third of their normal length and covered by columnar epithelial cells; the crypts of Lieberkühn are hyperplastic and coccidia are absent. HPS. X125.

Clinical signs of porcine neonatal coccidiosis are probably related to the severity and extent of the villous atrophy and cell replacement induced by the coccidia in the small intestine. These changes cause a maldigestion and malabsorption of nutrients because of a loss of the digestive-absorptive surface of the small intestine (25). Villous atrophy with secondary maldigestion and malabsorption has also been suggested as the pathogenesis of infection with *Eimeria acervulina* in chickens (26), *Eimeria crandallis* infection of lambs (26) and *Isospora ohioensis* in dogs (27). Hypersecretion caused by the crypt hyperplasia might also contribute to the diarrhea (25). Duration of the clinical disease is probably related to the time needed for villous regrowth and the possible residual effects caused by this infection have not been investigated. The multifocal

nature of the lesions could be an explanation for the low mortality rate in coccidiosis compared to that in TGE where the lesions are much more diffuse (22, 24). If mucosal ulceration becomes extensive and leads to a more or less severe fibrinonecrotic enteritis, the increased permeability of intestinal mucosa secondary to inflammation will also contribute to diarrhea and loss of proteins, and repair will be more difficult.

The prepatent period varied from four to seven days and the most common was five days. These observations are similar to those reported recently by Lindsay (20). Eighty percent of the piglets kept alive more than four days p.i. shed oocysts. The oocyst discharge began most frequently the same day diarrhea first appeared. Among the piglets dosed with oocysts less than two months old, the degree of oocyst shedding was a little higher in those piglets dosed with 50 000 oocysts (mean oocyst count was 15 150 compared to 10 800 in piglets dosed with 100 000 oocysts). This decrease in oocyst production related to larger infective doses of oocysts has also been reported by Stuart *et al* (19) in piglets. Larger doses of oocysts would induce more extensive lesions in the small intestine during the asexual phase leaving insufficient numbers of intact villi to support the gametogonic phase of the cycle. A similar phenomenon occurring in *Eimeria acervulina* infection of chickens has been called the "crowding effect" (28). It is also possible that interferon or an interferon-like substance produced in response to coccidia might intervene in this phenomenon (29). It appears also that the age of the inoculum might have an important influence on the oocyst discharge (30). In our study, the three piglets dosed with 100 000 old oocysts (inoculum ten month old) had a much higher mean oocyst count (320 000). A lower viability, infectivity or virulence of these oocysts causing less severe lesions during the asexual phase and then minimizing the "crowding effect" might explain this phenomenon. These observations on the influence of the dose and age of the inoculum on oocyst discharge might explain why in spontaneous cases of the disease, several piglets will shed very few oocysts while others will shed large numbers even in the absence of clinical signs (M. Morin; unpublished data 1982). It is well known in other species that oocyst shedding is not necessarily proportional to the severity of the clinical signs in coccidial infection (29). This shedding of oocysts by diseased or asymptomatic carriers stresses also the importance baby pigs probably have in maintaining the infection in farrowing houses.

Results of this work confirm *I. suis* as a cause of neonatal diarrhea in piglets. They also increase our knowledge of the pathogenesis of the infection and contribute towards a better understanding of its epizootiology which is essential for more efficient control of this important disease of neonatal piglets.

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