

TABLE OF CONTENTS

	Page Number
INTRODUCTION	1
LITERATURE REVIEW	3
Classification of Coccidia	3
Life Cycle of the Family Eimeriidae	4
Coccidia of Swine	5
Life Cycle of <u>Isospora suis</u>	7
Sporogony	7
Merogony and Gametogony	10
Patency	17
Pathogenicity of <u>Isospora suis</u>	17
Incidence of neonatal porcine coccidiosis	17
Clinical signs	18
Gross lesions	19
Histologic lesions	19
Diagnosis	20
Differential diagnosis	21
Experimental studies	21
MATERIALS AND METHODS	27
Experimental Animals	27
Inoculum	27
Experimental Design	27
Clinical Signs	29

	Page Number
Histopathology	30
Morphometric Evaluation of Villous Length and Crypt Depth	31
Scanning Electron Microscopy	33
Oocyst Counts	33
Evaluation for Other Gastrointestinal Pathogens	34
Statistical Analysis	34
RESULTS	37
Clinical Signs	37
Morphologic Lesions	38
Macroscopic lesions	38
Microscopic lesions	40
Morphometric Results	90
Oocyst Counts	92
Other Gastrointestinal Pathogens	92
DISCUSSION	142
SUMMARY	159
REFERENCES	162
ACKNOWLEDGEMENTS	168

INTRODUCTION

Isospora suis is now recognized as a significant cause of diarrhea in the baby pig. As early as 1934 H. E. Biester experimentally showed that Isospora suis produced clinical illness and lesions in baby pigs, but it was not until the mid to late 1970s that this coccidium was looked at as a major pathogenic organism.

Clinically the disease is associated with diarrhea in 5 to 14 day old pigs. The severity of signs is variable, ranging from retarded growth and unthriftiness to a severe diarrhea leading to dehydration, emaciation and death. Both morbidity and mortality are variable.

On gross post mortem examination lesions range from no lesions or a mild catarrhal enteritis to the often described fibrinonecrotic enteritis in the jejunum and ileum. A significant histopathologic lesion is villous atrophy, most severely affecting the mid-jejunum and ileum. There can be varying degrees of superficial epithelial erosion, mucosal necrosis and cellular infiltration. The severity of lesions depends upon the age and immune status of the pig at infection, the number of sporulated oocysts ingested, the stages in the parasite life cycle and the presence of concurrent enteric infection.

The objectives of this study were to experimentally infect piglets with Isospora suis and to:

- 1) describe the histologic lesions in the intestinal tract of infected pigs through the course of infection in comparison to those of age-matched uninfected controls.

- 2) determine the parasite distribution in the intestinal tract through the course of infection.
- 3) relate the histologic lesions and parasite distribution in the intestinal tract to the clinical course of infection and fecal shedding of oocysts.

LITERATURE REVIEW

Classification of Coccidia

Isospora suis is a protozoan classified, according to Levine (1985), within the Phylum Apicomplexa, Class Sporozoasida, Subclass Coccidiasina, Order Eucoccidiorida, Suborder Eimeriorina, and Family Eimeriidae. A characteristic feature of the Class Sporozoasida is a complex life cycle generally with an alternation of asexual and sexual reproduction with the sexual cycle resulting in the formation of oocysts that develop sporozoites. The Subclass Coccidiasina is distinguished by the formation of intracellular gamonts which are morphologically dissimilar (anisogamous). The life cycle may consist of merogony (asexual reproduction forming merozoites), gametogony (formation of gametes), and sporogony (formation of sporocysts and sporozoites by division of a zygote, the first division being by meiosis with subsequent divisions by mitosis). In the Order Eucoccidiorida both merogony and gametogony are present. There are four suborders within the Eucoccidiorida. The Eimeriorina are characterized by independent development of the macrogamont and microgamont and production of nonmotile oocysts that contain sporozoites enclosed in sporocysts. This suborder is divided into five families, three of which parasitize mammals: Eimeriidae, Cryptosporidiidae, and Sarcocystidae. Eimeriidae and Cryptosporidiidae complete their life cycles in one host whereas the family Sarcocystidae parasitizes both a definitive host and at least one intermediate host. Eimeriidae members develop within the host cell cytoplasm while Cryptosporidiidae members develop intracellularly but

extracytoplasmicly in the area of the brush border. There are five genera within the Family Eimeriidae, only two of which are important as parasites of domestic animals: Eimeria and Isospora. The separation into genera is based primarily on sporulated oocyst structure with Eimeria sp. oocysts containing four sporocysts each with two sporozoites and Isospora sp. oocysts containing two sporocysts each with four sporozoites.

There is some debate as to the definition of "coccidia". Some parasitologists would like to reserve the term for members of the Family Eimeriidae. Most taxonomists prefer to include all members of the Suborder Eimeriorina thereby adding, among others, Sarcocystis, Toxoplasma, Hammondia, Besnoitia, and Frenkelia to the list of coccidia. With the discovery of the complex life cycle of Toxoplasma in cats and other dual host life cycles in species with an Isospora-type oocyst controversy has arisen as to the validity of some of the separations made between the families Sarcocystidae and Eimeriidae (Levine et al., 1980).

Life Cycle of the Family Eimeriidae

The similarities between the Isospora and Eimeria genera have long been recognized. They are usually both monoxenous and stenoxenous, i.e., their life cycles are completed within one host, and each species is specific to one host species. Both genera develop through the classical coccidial life cycle. A sporulated oocyst is ingested. The sporozoites, the motile infective stages, excyst in the gastro-intestinal tract of the host. Three stimuli are generally needed for excystation: CO₂, trypsin and bile salts. The sporozoite penetrates the intestinal epithelial cell

to become a meront, an asexual stage that forms merozoites by multiple fission. The merozoites break out of the infected epithelial cell, usually destroying the cell, and penetrate another epithelial cell to form the second generation meront. This continues for a coccidia-species specific number of meront generations at which time the infective merozoite forms a gamont, a cell that will develop into either a macrogamont (a uninuclear gamont that forms a large single female gamete) or a microgamont (a gamont that produces multiple small male gametes). The microgametes are released from the cell. One microgamete penetrates and fertilizes a macrogamete to form a zygote. A wall develops around the zygote to form an oocyst. The oocyst breaks out of the epithelial cell and is shed in the feces of the host. In the process of sporulation, the sporont divides to form sporoblasts which mature to form sporocysts; the protoplasm inside each sporocyst divides to form the sporozoites. The time required for sporulation to the infective stage is species specific although oxygen, adequate moisture and optimum temperatures influence the process.

Coccidia of Swine

Several species of coccidia have been identified in swine, few have been associated with disease.

Levine (1985) listed eleven Eimeria species that infect swine:

Eimeria deblickei Douwes, 1921

Eimeria suis Noller, 1921

Eimeria perminuta Henry, 1931

Eimeria scabra Henry, 1931

Eimeria spinosa Henry, 1931

Eimeria polita Pellerdy, 1949

Eimeria porci Vetterling, 1965

Eimeria neodebliecki Vetterling, 1965

Eimeria guevarai Romero and Lizcano, 1971

Eimeria betica Martinez and Hernandez, 1973

Eimeria residualis Martinez and Hernandez, 1973

Levine also mentioned Eimeria sp. Desser, 1978 that was found in the bile duct epithelium of the liver of the domestic pig in New Zealand.

Soulsby (1982) listed eleven species of swine Eimeria as well; however he omitted E. betica and E. residualis and added Eimeria cerdonis Vetterling, 1965 (which Levine claimed is synonymous with E. polita) and Eimeria scrofae Galli-Valerio, 1935 (which Levine claimed is synonymous with E. debliecki). Pellerdy (1974) accepted nine species of Eimeria in pigs omitting E. suis, E. betica and E. residualis and adding Eimeria romaniae to the list. Both Fayer (1980) and Vetterling (1965) listed eight Eimeria omitting E. polita, E. guevarai, E. betica and E. residualis and adding E. cerdonis.

The most common coccidium found in swine is reported to be E. debliecki (Soulsby, 1982). This coccidium parasitizes the jejunum and ileum. Pathogenic effects are reported in young pigs (Levine, 1985). Heavy infections have caused clinical signs of diarrhea and emaciation. E. scabra parasitizes epithelium of the jejunum and ileum (Soulsby, 1982). A few reports indicated that this coccidium may be pathogenic causing

diarrhea and enteric lesions (Soulsby, 1982; Levine, 1985). E. spinosa and E. polita are common coccidia that infect the epithelial cells of the small intestine. Both may have the pathogenic capability of producing diarrhea (Levine, 1985). Little is known of the other Eimeria; in fact many of these species are known only by their oocysts.

As for Isospora coccidia of swine, Levine (1985) listed three species: Isospora suis Biester, 1934; Isospora almataensis Paichuk, 1953; and Isospora neyrai Romero and Lizcano, 1971; as well as an unnamed species described by Shrivastava and Shah, 1968. Little is known about the last three species. Levine strongly suggested that all but Isospora suis may be pseudoparasites in the pig. It is Isospora suis that has sparked much interest in recent years. The following discussion of the literature is restricted to this parasite.

Life Cycle of Isospora suis

The life cycle of Isospora suis has been studied and described most extensively by Biester and Murray (1934), Vetterling (1965), Cesario (1980), Matuschka and Heydorn (1980), Lindsay et al. (1980), and Harleman (1982) (Table 1).

Sporogony

The fully sporulated oocyst of Isospora suis contains two ellipsoidal sporocysts each of which contains four sporozoites. Biester and Murray (1934) described the oocyst as having a smooth yellow-brown, double-layered wall and as having a polar inclusion within the oocyst. Vetterling (1965)

Table 1. Comparative Results of Studies
on the Life Cycle of *Isospora suis*
Modified from Harleman (1982)

Source	Oocyst Size Average (μm .)	Oocyst Wall Characteristics
Biester and Murray (1934)	22.5 x 19.4 ^a	smooth, yellow-brown, double walled. 1.5 μm . ^a , 0.66 μm . ^b
Vetterling (1965)	19.5 x 17.3 ^b	smooth, colorless, single walled, 0.5 - 0.7 μm . ^b
Cesario (1980)	22.0 x 16.4 ^b	smooth, colorless, single walled, 0.8 μm . ^b
Matuschka and Heydorn (1980)	20.2 x 16.9 ^a 21.5 x 18.8 ^b	smooth, colorless, single walled, 1.0 μm . ^b
Lindsay et al. (1980)	21.2 x 18.4 ^b	_____
Harleman (1982)	_____	_____
Robinson and Morin (1983)	21.2 x 19.9 ^b	_____
Ernst et al. (1986)	_____	_____

^aMeasurements obtained from unsporulated oocysts.

^bMeasurements obtained from sporulated oocysts.

^cDescribes biphasic fecal oocyst shedding.

Sporocyst Size Average (um.)	Prepatent Period (hrs.)	Patent Period (days)	Sporulation Time (days)
16.4 x 11.2	148 - 192	7	4
13.0 x 9.3	120	3	4
14.3 x 10.9	108	3	4
15.5 x 11.0	132-144 ^C	10-18	1-2 @ 25 ⁰ C
14.5 x 10.3	120	5-8	2.33 @ 20 ⁰ C to 0.5 @ 37 ⁰ C
_____	110 & 264 ^C	3-4	_____
_____	_____	_____	_____
_____	_____	_____	1 - 2 @ 25 ⁰ C

described the oocyst with a smooth, colorless single-layered wall. He saw no polar inclusion inside the sporulated oocyst. Lindsay et al. (1980) noted 2-4 um hazy bodies present between the sporont and the oocyst wall in the unsporulated oocyst. Cesario (1980) and Matuschka and Heydorn (1980) described the oocyst with a smooth, colorless single-layered wall. All agreed that the Isospora oocyst lacked evidence of a micropyle, an oocyst residuum and a Stieda body.

The sporulation time has often been recorded as four days (Biester and Murray, 1934; Vetterling, 1965; and Cesario, 1980). Matuschka and Heydorn (1980) and Ernst et al. (1986) reported a sporulation time of 24-48 hrs. at 25°C. Lindsay et al. (1982) reported different sporulation times as temperature varies: 56 hrs. @ 20°C, 40 hrs. @ 25°C, 16 hrs. @ 30°C, and 12 hrs. @ 37°C with cessation of sporulation at temperatures of 40°C or above. They pointed out that a common management procedure in farrowing houses is to keep temperatures between 32°C and 35°C, hence encouraging short sporulation times.

Merogony and Gametogony

Histologic studies of the endogenous stages of Isospora suis have been carried out (Table 2). Biester and Murray (1934) experimentally infected four 80 lb. pigs. At six days post inoculation (pi) they found merozoites in the distal duodenum, jejunum, and ileum. At nine days pi mucosal scrapings of the small intestine revealed merozoites and macrogametes as well as oocysts. At eleven days pi mucosal scrapings of the small intestine had no Isospora although rectal contents still contained oocysts.

Cesario (1980) studied Isospora suis in gnotobiotic piglets in two trials. In the first trial he fed each of six 6-day old piglets 5,000 sporulated oocysts, and necropsied the pigs at 24, 48, 72, 96, 120, and 144 hrs. pi. In the second trial he inoculated four 8-day old pigs, and necropsied them at 24, 48, 72, and 120 hrs. pi. No endogenous stages of Isospora suis were seen until 96 hrs. pi. Meronts were seen in the epithelial cells of the distal two-thirds of the jejunal and ileal villi. He noted that the meronts tended to avoid the vacuolated epithelial cells normally present on the distal four-fifths of intestinal villi in neonatal piglets. Meronts were not seen in the crypts of Lieberkuhn. Within their parasitophorous vacuoles in the cell, most meronts displaced the host cell nucleus apically. Two to twenty-five merozoites were observed within the meronts. The merozoites were banana-shaped, each with a central nucleus.

A small number of immature gamonts was present at 96 hrs. pi. The peak number of mature gamonts was seen at 120 hrs. pi, with sexual forms present in every section of intestinal tissue, duodenum through colon. The sexual stages were rare in the duodenum, cecum, and colon and most numerous in the posterior jejunum and anterior ileum. The gamonts were generally in epithelial cells of the distal three-fourths of the villi with apparently no preference for the nonvacuolated epithelial cells. No sexual stages were seen in the crypts of Lieberkuhn. The gamonts appeared in parasitophorous vacuoles proximal to the host cell nucleus. Macrogametocytes outnumbered microgametocytes in a ratio of 15:1. Microgametocytes were observed to undergo an intricate process of

Table 2. Comparative Results of Endogenous Stages in the Life Cycle of Isospora suis

ASEXUAL					
Source	Type	Size of Meront (um.)	Number of Merozoites	Size of Merozoites (um.)	Day 1st Observed (days pi)
Cesario (1980)	_____	16.6 x 11.9	2 - 25	9.2 x 3.2	4
Matuschka and Heydorn (1980)	First sitings	_____	_____	18.9 x 4.7	2
	Second sitings				
	A.	19.6 x 6.3	4 - 16	16.0 x 4.0	4
	B.	12.6 x 3.1		9.5 x 2.5	
Lindsay et al. (1980)	Type I	10.4 x 4.7	2 - 14	10.0 x 3.6	3
	Type II	11.4 x 5.3	2 - 16	6.3 x 2.1	4
Harleman (1982)	First generation	9.0 x 5.0	2 - 7	9.8 x 5.3	1
	Second generation	_____	2 - 12	13.4 x 5.5	3
	Third generation	_____	4 - 22	8.0 x 2.8	4
	_____	_____	_____	12.7 x 4.7	8

 SEXUAL

Size of Macro- gametocyte (um.)	Size of Micro- gametocyte (um.)	Size of Micro- gamete (um.)	Day 1st Observed (days pi)
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14.6 x 12.3	19.2 x 13.5	3.8 x 7	4
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_____	_____	_____	4
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9.4 x 6.5	11.8 x 8.4	3 - 5	4
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_____	_____	_____	_____
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development. They began as a spherical body with many nuclei evenly dispersed. These nuclei migrated to the periphery of the cell. The nuclei matured to form biflagellated microgametocytes.

Lindsay et al. (1980) inoculated nine 1-day old pigs with 150,000 sporulated oocysts each and necropsied them at 12, 24, 36 hrs. pi and 2, 3, 4, 5, 6, or 7 days pi. They also inoculated five 1-day old pigs with 200,000 sporulated oocysts each, and necropsied the pigs at 8, 10, 12, and 14 days pi. No organisms were seen until 36 hrs. pi when uninucleated zoites were observed in mucosal scrapings from the jejunum and ileum. Endogenous stages were not seen in tissue sections until three days pi at which time meronts were seen. Lindsay et al. (1980) called these meronts "Type I". The meronts were either binucleated or contained single or multiple (2-14) merozoites. At four days pi intense asexual reproduction was observed. It was at this time that the "Type II" meronts were first noticed being larger than the Type I meront and containing smaller merozoites. They were multinucleated (3-12 nuclei) and occurred either singly or in groups of two to four to a cell. The Type II uninucleated merozoites were in groups of two to sixteen, often attached to each other at one end. At four days pi the distribution was estimated to be 53% Type I and 26% Type II meronts with 21% Type II merozoites. At five days pi the distribution was 17% Type I, 30% Type II meronts, 24% Type II merozoites and 29% sexual stages.

Sexual stages were not seen until four days pi. At five days pi the 29% sexual stages were comprised of 1% microgamonts, 25% macrogamonts and

3% oocysts. At six days pi the distribution was 5% asexual stages, 24% microgamonts, 57% macrogamonts, and 14% oocysts. No sexual stages were seen at twelve or fourteen days pi.

Matuschka and Heydorn (1980) infected 20 piglets (7 were 12-24 hours old, and 13 were 18-21 days old) with doses of 10^3 , 10^5 , or 10^6 sporulated Isospora suis oocysts each. They necropsied the infected piglets at 24, 48, 72, 96, 120, 144 and 168 hrs. pi. At 24 hrs. pi many zoites had invaded villous epithelial cells with the maximum concentration being found in the anterior one-third of the jejunum. At 48 hrs. pi the number of merozoites observed had increased. At 72 hrs. pi the number of parasites had greatly increased. Many merozoites were binucleated. At 96 hrs. pi two different sized schizonts were observed. The larger meronts were more numerous. Mature meronts contained 4-16 merozoites. Asexual multiplication was shown to begin with endodyogeny (the formation of two daughter cells by internal budding).

The first sexual forms were seen at 96 and 120 hrs. pi. The first young oocysts were identified at 120 hrs. pi. At 144 hrs. pi the predominant parasite stages were gamonts and unsporulated oocysts with the highest concentration centering around the posterior half of the small intestine. There was a notable decrease in gametogony with primarily mature gamonts and oocysts present in the intestinal epithelium. No developmental stages were ever seen in the cecum or colon.

Harleman (1982) inoculated fourteen 2-day old germfree pigs and seven 2-day old conventionalized pigs each with 240,000 sporulated Isospora suis

oocysts and necropsied the pigs at 3, 4, 5, 6, 8, 9, 10, 11, and 14 days pi. The early stages of the life cycle were studied in four gnotobiotic pigs infected with 5×10^7 sporulated oocysts each and necropsied at 24 and 48 hrs. pi.

At 24 hrs. pi numerous merozoites were seen within the villous epithelium. Usually only one meront existed within a parasitophorous vacuole. The meronts were characterized by a small clear vacuole proximal to the nucleus. At 48 hrs. pi many first generation meronts with 2-7 merozoites were seen. At 3 days pi the first generation meronts were still present although the second generation meronts predominated. These meronts contained 2-12 large merozoites. This stage, although most common at three to four days pi, was still present at 6 days pi. A third generation of meronts was seen at day 4 pi. These meronts contained 4-22 small merozoites. Some of these merozoites were attached to a residual mass. This generation occurred 4 to 6 days pi with its peak at 4 to 5 days pi. Harleman believed his 2nd and 3rd generation meronts were similar to the single asexual stage described by Cesario (1980) and the two types of meronts described by Lindsay et al. (1980).

Sexual stages were seen from 4 days pi to 8 days pi being most numerous at 5-6 days pi and coinciding with oocyst excretion. At 8 to 10 days pi asexual stages were seen again with the meronts and merozoites resembling the second generation. These forms were present in crypt epithelial cells and in Brunner glands. The following sexual cycle occurred at 11 and 14 days pi which again tended to locate more frequently

in crypt epithelium.

Patency

Among the investigators there is some disagreement as to the prepatent and patent period lengths. The prepatent period was reported to be 148-192 hours by Biester and Murray (1934), 132-144 hours by Matuschka and Heydorn (1980), 120 hours by both Vetterling (1965) and Lindsay et al. (1980), and 108 hours by Cesario (1980). Matuschka and Heydorn (1980) reported a biphasic fecal shedding pattern with the first peak at seven to ten days pi and the second peak at thirteen to fifteen days pi. Harleman (1982) described a biphasic patency with the first appearance of oocysts occurring at five days and the second appearance of oocysts occurring at eleven days. Reported patency periods include ten to eighteen days (Matuschka and Heydorn, 1980), seven days (Biester and Murray, 1934), five to eight days (Lindsay et al., 1980), three to four days (Harleman, 1980), and three days (Vetterling, 1965 and Cesario, 1980).

Pathogenicity of Isospora suis

Incidence of neonatal porcine coccidiosis

From 1934 to 1976 reports on the possible pathogenicity of Isospora suis occurred sporadically in the literature. As swine management practices changed towards total confinement and continuous farrowing, a diarrhea problem in suckling pigs associated with the presence of Isospora suis emerged. Between 1977 and 1983 a steady increase in the number of Isospora associated baby pig diarrheas was noted at numerous diagnostic laboratories throughout the United States and Canada (Bergeland,

1977; Sangster et al., 1978; Stuart et al., 1980b; Clark, 1980; Brokken, 1980; Morin et al., 1980; Sanford and Josephson, 1981; Eustis and Nelson, 1981; Hoefling, 1981; Stevenson and Andrews, 1982; Sanford, 1983).

Many authors reported a significant increase in cases of neonatal coccidiosis in the summer and fall months (Brokken, 1980; Stuart et al., 1980b; Morin et al., 1980; Lindsay et al., 1983; Sanford, 1983). Many reports referred to the incidence increasing with both continuous farrowing practice and total confinement units (Hoefling, 1978; Morin et al., 1980; Roberts and Walker, 1981; Meyer, 1982; Lindsay et al., 1983; Sanford, 1983), although as Lindsay et al. (1983) pointed out the disease can be seen in nearly any swine rearing situation.

Clinical signs

During these years of recognition of the Isospora problem there were numerous reports on the clinical history, clinical signs, gross post mortem findings, and histologic lesions found with the disease. Most reports were in general agreement. The diarrhea was usually sudden in previously healthy piglets. The piglets ranged between 5 and 14 days of age with the highest incidence occurring in 7 to 10 day old pigs. The earliest report was in a 3 day old pig (Hoefling, 1978). The diarrhea was acute in onset and the fecal discharge was yellow and watery. As the diarrhea continued the pigs became dehydrated, anorexic and emaciated. Vomiting was rarely reported. With time some pigs developed a long hair coat and appeared unthrifty. Some severely affected pigs grew poorly. Others apparently recovered. Morbidity was variable; mortality was generally less than 20%

although Meyer (1982) reported an outbreak with a mortality rate of 40 to 90%. The diarrhea problem was often persistent on the farm, lasting months and affecting successive litters.

Gross lesions

Gross lesions included fluid, yellow intestinal contents often with a catarrhal exudate (Sanford and Josephson, 1981). The most striking gross lesion noted was a fibrinonecrotic adherent membrane most often covering the posterior jejunum and anterior ileum (Sanford and Josephson, 1981). There was wide variation in the reports on the incidence of this membrane: 33% (Bergeland, 1972), 26% (Eustis and Nelson, 1981), 10-20% (Stuart et al., 1980a), 8% (Stevenson and Andrews, 1982; Sangster et al., 1978; Sanford and Josephson, 1981) and 5% (Robinson and Morin, 1982). Sanford (1983) noted that the incidence of adherent membranes increased from 8% in 1980 to 17% in 1982.

Histologic lesions

The histologic lesion was primarily a mild to severe villous atrophy with accompanying crypt hyperplasia (Bergeland, 1977; Sangster et al., 1978; Morin et al., 1980; Roberts, 1980; Roberts et al., 1980; Eustis and Nelson, 1981; Sanford and Josephson, 1981; Robinson and Morin, 1982). The most severe changes were seen in the posterior jejunum and anterior ileum. A multifocal necrotic enteritis was seen in the more severely affected pigs (Sangster et al., 1978; Eustis and Nelson, 1981; Sanford and Josephson, 1981; Stevenson and Andrews, 1982). Occasionally fusion of villi was noted (Sanford and Josephson, 1981). The most common form of the

parasite noted in tissue sections of affected piglets was the meront/merozoite (Sangster et al., 1978; Morin et al., 1980; Sanford and Josephson, 1981; and Robinson and Morin, 1982). These forms were primarily seen in the epithelium covering the distal half of the jejunal and ileal villi although the duodenum (Robinson and Morin, 1982), colon (Robinson and Morin, 1982; Sanford, 1983) and rarely the spiral colon (Sangster et al., 1978) were parasitized. A few reports mentioned the invasion of crypt epithelium (Robinson and Morin, 1982; Sanford, 1983). An inflammatory response in the lamina propria, including an infiltration of lymphocytes, plasma cells (Sangster et al., 1978; Eustis and Nelson, 1981; Sanford and Josephson, 1981; Robinson and Morin, 1982), eosinophils (Sangster et al., 1978; Sanford and Josephson, 1981), and neutrophils (Eustis and Nelson, 1981; Stevenson and Andrews, 1982) has been documented.

Diagnosis

The diagnosis of Isospora suis neonatal diarrhea in pigs was most often confirmed via histopathologic examination of the jejunum and ileum. However, to aid in the speed and ease of field diagnosis some authors have made a few suggestions. A typical history and typical clinical signs in the piglets were major clues to the diagnosis. Necropsying a few of the affected pigs and finding an adherent fibrinonecrotic membrane over the jejunum was highly suggestive of coccidiosis. Some authors (Stevenson and Andrews, 1982) recommended mucosal impression smears, claiming that there was a high correlation of the number of parasites seen per high power field in a smear with the number of organisms seen per high power field in a

corresponding tissue section. Sanford (1983) believed that mucosal scrapings were more reliable than impression smears. Most agreed that fecal examinations of clinically affected animals were not reliable for a diagnosis. Sanford (1983) claimed only 7% of the diagnosed coccidial infections were positive on fecal exam. In contrast, however, Stuart (1982) claimed that 50% of the piglets diagnosed were positive by fecal exam.

Differential diagnosis

The differential diagnosis for diarrhea in a 7 to 14 day old piglet must include transmissible gastroenteritis (TGE) virus, rota virus enteritis, Clostridium perfringens enteritis, and Escherichia coli enteritis (Lindsay et al., 1983). TGE virus and rota virus infection will produce villous atrophy with a mild to marked enteritis. Clostridium perfringens produces a toxin which causes a severe necrotic enteritis. Strains of E. coli capable of causing a diarrhea attach to the epithelial cells and produce an enterotoxin which stimulates secretion with no apparent villous atrophy.

Experimental studies

Experimentally researchers have found clinical signs and gross and microscopic lesions in piglets they have inoculated with Isospora suis oocysts similar to those clinical signs and lesions described in field cases. Matuschka and Heydorn (1980), after dosing piglets with 10^6 sporulated Isospora suis oocysts each observed clinical signs including vomiting at 3-6 hrs. pi, severe watery diarrhea at 3 days pi (occasionally

with blood) that became intermittent at 7 days pi, abdominal pain and a marked decrease in appetite at 3-7 days pi. There was a high mortality rate at 3-5 days pi in piglets infected at 12-24 hrs. of age.

Histologically the small intestinal villi were damaged as evidenced by sloughing epithelium and broad stunted villi. Matuschka and Heydorn (1980) showed that Isospora suis infected piglets had markedly lower weight gains and feed efficiencies as compared to control animals. At two weeks pi the infected piglets had 40% less weight gain. At four weeks pi the infected piglets still showed 14% less weight gain.

Matuschka and Heydorn searched for extraintestinal stages of Isospora suis. They dosed one piglet orally with 10^6 sporulated Isospora suis oocysts, homogenated its liver, spleen, mesenteric lymph nodes and muscle 29 days later and orally inoculated this mixture into two piglets. They also orally dosed two mice each with 10^5 sporulated Isospora suis oocysts, homogenated organs from the mice 3 to 7 days later and fed this mixture to a piglet. The dosed piglets' feces were checked daily for Isospora suis oocysts over a three week period; none were found. The piglets were then necropsied and their organs and intestinal tracts examined for the presence of Isospora suis parasites; none were found.

Stuart et al. (1980a) inoculated two litters of 3-day-old pigs with 3,000 and 100,000 oocysts per pig. Three other litters of 1-day old pigs were inoculated with 150,000; 200,000; and 400,000 sporulated oocysts per pig respectively. No gross and only minimal histologic changes were seen in piglets inoculated with 3,000 and 100,000 oocysts. The piglets dosed

with 150,000 or more oocysts generally developed diarrhea 72 hrs. after inoculation. The diarrhea generally lasted 2-6 days depending on the dose of inoculum. At 4-6 days pi prominent villous atrophy was seen in the jejunum and ileum with mild villous atrophy in the duodenum. Moderate numbers of asexual and sexual stages were noted at this time through the small intestine and occasionally within the colon. Fibrinonecrotic membranes were found in the more severely affected piglets dosed with 200,000 oocysts and necropsied at 5 days pi or 10 days pi. Piglets dosed with 400,000 oocysts were dramatically affected. Two died by 72 hrs. pi, four died by 96 hrs. pi, and four others were comatose at 96 hrs. pi. On necropsy, six of twelve piglets had adherent fibrinonecrotic membranes. There were marked villous atrophy, crypt hyperplasia, villous necrosis, and lymphocytic and neutrophilic infiltrates. Both asexual and sexual stages were seen from the duodenum to the colon.

Stuart et al. (1982a) showed a dose and age response of piglets to Isospora suis. They compared lesions and clinical signs in pigs infected with Isospora suis at 1 to 3 days of age to those seen in pigs infected at two weeks of age. A dose of 200,000 oocysts caused severe diarrhea at 3 to 4 days pi in one day old pigs. Two out of nine of these piglets developed fibrinonecrotic membranes over the jejunum and ileum. Piglets dosed with 400,000 oocysts at one day of age developed a severe diarrhea leading to a comatose state by 3 to 5 days pi. Nine of seventeen of these pigs developed a fibrinonecrotic membrane. Piglets dosed with 400,000 oocysts at two weeks of age developed a mild diarrhea at 3 to 4 days pi with only

mild histologic lesions. In piglets that were four weeks old a dose of 2,000,000 oocysts produced a mild diarrhea and no significant microscopic lesions.

Robinson et al. (1983) dosed 28 three day old pigs with either 50,000 or 100,000 sporulated oocysts per pig and necropsied them between 3 and 12 days pi. Diarrhea commenced 4 to 5 days pi. Villous atrophy seemed related to the destruction of villous epithelium during peak asexual reproduction at 4 to 5 days pi. Microscopic lesions were restricted to the small intestine. The sexual phase of the cycle seemed to peak at 5 days pi.

In 1982 Meyer reported on his studies of Isospora suis in gnotobiotic swine. He found that diarrhea began at 120 hrs. pi. Cesario (1980), working with Meyer, reported on gnotobiotic piglets infected with Isospora suis and stated that diarrhea began at 140 hrs. pi. Few histopathologic changes were noticed in the gnotobiotic animals. The most severe lesion seen was villous atrophy with little inflammatory reaction and no necrosis evident. His results suggested that the intestinal microflora contributes to the development of extensive lesions. He also related the onset of clinical signs more closely with the appearance of large numbers of sexual stages and the rupture of the intestinal cells releasing oocysts.

Harleman (1982) studied Isospora suis in both gnotobiotic and conventional piglets. He inoculated 2 to 3-day old piglets each with 240,000 sporulated oocysts. At one to two days pi he noted mild villous atrophy and necrosis associated with high numbers of parasites in both

groups. At three days pi diarrhea began in the conventional piglets. Most conventional pigs dosed with 240,000 oocysts died at four days pi. By four to six days the gnotobiotic piglets began to scour. Marked villous atrophy and necrosis were present in both conventional and gnotobiotic animals. By six to eight days pi the animals' condition improved. At eight to ten days pi diarrhea returned or worsened. Histologically, marked villous atrophy and epithelial necrosis were noted again associated with the appearance of asexual forms of coccidia.

With these findings Harleman proposed the possibility of extraintestinal development of Isospora suis. This stage may be associated with lesions Harleman reported within the mesenteric lymph nodes (lymphoid hyperplasia) and the liver (infiltrates of neutrophils, focal areas of necrosis and cholestasis). Further investigations in an attempt to find evidence of extraintestinal stages were performed by Harleman. He made homogenates of the mesenteric lymph nodes, spleen and liver from Isospora suis infected pigs killed at 24 and 48 hrs. pi. These homogenates were injected into the peritoneal cavity of Isospora-free piglets. These piglets began shedding Isospora oocysts at 11-12 and 16-17 days pi.

Other studies have attempted to find extraintestinal stages of Isospora suis. No one has yet found the organism in any extraintestinal tissues. Stuart et al. (1982b) noted focal pyogranulomatous lymphadenitis within the mesenteric lymph nodes. They attempted to find evidence for extraintestinal stages by feeding Isospora-free piglets a homogenate of mesenteric lymph node and spleen from an Isospora infected piglet. The

homogenate-fed piglets failed to pass Isospora oocysts.

In order to determine the effect of Isospora suis on morbidity, mortality and weight gain Lindsay, Current and Taylor (1985) experimentally infected 49 piglets at 3 days of age with 300,000 sporulated oocysts and compared them to 51 control piglets. Diarrhea began at 3-5 days p.i. and lasted generally from 6-10 days. Microscopic lesions included villous atrophy and necrotic enteritis primarily located within the jejunum and ileum and associated with the asexual stages of Isospora suis. Four pigs died at 4-5 days pi; five pigs died at 11-13 days pi. The overall mortality rate was 20.4%. Differences between control and infected piglets were noted in their weight gains during the first 2 weeks of life. The difference between the two groups in weight gain between 2 weeks and 3 weeks of age was not significant, even though infected piglets still weighed much less than the 3-week old control piglets.

MATERIALS AND METHODS

Experimental Animals

Seventy-eight pigs from eight crossbred sows were used in this study. The sows with their respective litters of piglets were housed indoors in facilities at Iowa State University.

Inoculum

A culture of Isospora suis obtained in 1981 from pigs in a naturally infected herd in Iowa was maintained by passage in neonatal pigs. Oocysts were sporulated at 28°C for 2-3 days in 2.5% aqueous potassium dichromate and stored at 4°C. For inoculation sporulated oocysts were washed free of dichromate, enumerated and suspended at a known concentration in tap water.

Experimental Design

Thirty-nine piglets from four litters (L377, L379, L708, L738) remained uninfected and were used as controls. Forty-three piglets from the other four litters (L378, L384, L728, L999) were each infected with approximately 70,000 sporulated oocysts via stomach tube at 36-38 hours of age (Table 3).

From both the control and infected groups one or two pigs were euthanized at 3, 6, 10, 16, 26 and 36 days of age corresponding to 2, 5, 9, 15, 25 and 35 days post-inoculation (Table 3). Hereafter, days pi will be used to denote the age of piglets from both control and infected litters. The pigs were induced into a surgical plane of anesthesia with IV pentobarbital (Nembutal^R)¹ via a cranial vena caval puncture. Although some pigs died during the procedures most were sacrificed after all intestinal sections were removed.

¹Abbott Laboratories, North Chicago, Illinois

Table 3: Experimental Design

Group	Litter	Number of pigs in study	Number of pigs necropsied					
			2 days pi	5 days pi	9 days pi	15 days pi	25 days pi	35 days pi
Control	377	11	2	2	2	2	2	1
	379	10	2	2	2	2	2	0
	708	10	2	2	2	2	2	0
	738	8	2	2	2	1	1	0
	Total	39	8	8	8	7	7	1
Infected	378	9	2	2	2	2	1	0
	384	10	2	2	2	2	2	0
	728	12	2	2	2	2	2	2
	999	8	2	2	2	1	1	0
	Total	39	8	8	8	7	6	2

Unfixed sections were taken for TGE virus exam from the ileum and for bacterial culture from the duodenum, jejunum and ileum. Sections for formalin fixation were taken from nine locations of intestine. Three sections of intestine from each location were fixed in 10% neutral buffered formalin in the following manner. The first approximate 5 cm section of intestine was tied off at both ends and filled intraluminally with formalin and then immersed in formalin. The adjacent approximate 3 cm of intestine was simply placed in formalin while the next approximate 4 cm section was opened longitudinally and stapled to an acetyl sheet before being fixed in formalin. The nine locations of intestine were identified as follows:

duodenum: beginning just caudal to the pylorus.

jejunum-100: beginning 100 cm from the pylorus.

jejunum-200: beginning 200 cm from the pylorus.

lower-jejunum: beginning 150 cm proximal to the ileocecal junction.

ileum-15: beginning 15 cm proximal to the ileocecal junction.

ileum-5: beginning 5 cm proximal to the ileocecal junction.

cecum: beginning mid-body to the apex of the cecum.

proximal colon: beginning just caudal to the cecum.

spiral colon: centered about the ansa centralis.

Clinical Signs

All pigs were observed twice daily for clinical signs of illness. Notations were recorded on general appearance and attitudes of piglets; onset, character and duration of diarrhea; estimated amount of dehydration; occurrence of vomiting, and mortality.

Histopathology

Tissues fixed in formalin for at least 48 hours were trimmed and processed by routine paraffin techniques, sectioned at 5um and stained with hemotoxylin and eosin (H & E). The H & E sections from each of the nine locations of intestine were evaluated for mucosal epithelial changes, villous atrophy, villous fusion, and inflammatory infiltrates both within the epithelium and within the lamina propria.

Cuboidal metaplasia of the small intestinal epithelial surfaces was subjectively evaluated by scoring the relative amounts of columnar and cuboidal epithelium covering the villi and crypts. The scoring system was:

<u>State of Epithelium</u>	<u>Cuboidal</u>	:	<u>Columnar</u>
entirely columnar	0		4+
tips of villi cuboidal	1+		3+
1/2 cuboidal, 1/2 columnar	2+		2+
columnar cells at base	3+		1+
entirely cuboidal	4+		0

In the most severely affected areas another category, squamous metaplasia, was added in order to include an evaluation of squamous metaplasia in the villous epithelium. Villous fusion and focal necrosis were estimated with scores ranging from (0) to (4+). (0 = none, 1+ = slight, 2+ = mild, 3+ = moderate, 4+ = severe) Estimates of villous length/crypt depth ratios were made during histologic examination while exact measurements of whole villi and crypts were

determined in three small intestinal sections by morphometric techniques described later.

Ten full length villi and twenty crypts were evaluated for enumeration of intraepithelial lymphocytes, neutrophils and eosinophils, and goblet cells. Mitotic figures were counted in twenty crypts and identified as the mitotic index. Neutrophils and eosinophils within the lamina propria were counted in five high powered fields, averaged and recorded. The number of lymphocytes, plasma cells and macrophages within the lamina propria were subjectively evaluated using scores from (0) to (4+). (0 = none, 1+ = few, 2+ = some, 3+ = many, 4+ = abundant)

All the parasites in the entire formalin filled section from each location of intestine were counted and identified as to stage of development (asexual meront, microgametocyte, macrogametocyte, oocyst or unidentified parasite). Special attention was paid to the domes, the specialized intestinal structures consisting of lymphoid aggregates in the lamina propria and submucosa of the intestine that are important in the development of mucosal immunity. Domes were evaluated for epithelial changes and for the presence of parasites, goblet cells and inflammatory cells.

Morphometric Evaluation of Villous Length and Crypt Depth

Villous length and crypt depth were measured to evaluate villous atrophy, crypt hyperplasia and the villous/crypt ratios. Measurements were taken from three locations of intestine (duodenum, jejunum-200

and ileum-15) from all pigs in 3 control litters (L377, L708 and L738) and all pigs in 3 infected litters (L378, L384 and L728). In order to standardize the measurements of the sections, a Vibratome 1000¹ was used to cut the tissues. A flat section of formalin-fixed small intestine was trimmed with a razor blade to a 2 x 5 mm rectangle. One of the 5 mm cut surfaces was mounted on a specimen block with cyanoacrylate adhesive (Duro Superglue^R)². The block was then clamped in the vibratome tank and covered with 0.02 M sodium phosphate buffer, pH=7.0 (PB). In order to obtain a straight 90° cut surface, a single edged razor blade was mounted on the vibratome at a 15° angle. Several sections from each block were cut 80 um thick. The sections were placed on a microscope slide in a few drops of PB. A drop of 1% methylene blue in PB was added. A coverslip was then placed on the slide. The sections were examined under a light microscope equipped with a graticule in the 10x eyepiece which had been calibrated with the microscope's 10x objective lens for conversion of all measurements into microns. The lengths of twenty intact villi and the depths of ten intact crypts were measured from each preparation. The measurements were converted to microns. The average of the twenty villi and ten crypts was recorded and used to calculate the villous/crypt ratio.

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1. Technical Products International, Inc; St. Louis, Missouri
 2. Loctite Corporation, Cleveland, Ohio

Scanning Electron Microscopy

Formalin fixed material was prepared for scanning electron microscopy (EM). The tissues were cut from the formalin-filled loops and placed in a glutaraldehyde solution with 3% glutaraldehyde, 8% tannic acid in 0.1M cacodylate buffer, pH=7.4 (CB) and kept at room temperature on a rotator overnight. The tissues were then rinsed with CB for 15 minutes twice. The tissues were placed in a 0.5% OsO₄ solution in fresh CB for 30 minutes on a rotator and then rinsed six times with fresh distilled water over a 15 minute period. The tissues were placed in a 5% tannic acid/distilled water solution for one hour on a rotator and then rinsed quickly three times in distilled water. The tissues were placed in a 0.5% OsO₄ solution in distilled water for one hour on a rotator then rinsed with distilled water six times over a 15 minute period. The tissues were dehydrated by placing them successively in the following solutions for 15 minutes each: 50%, 75%, 95% and 100% ethanol; 3:1 ethanol/freon, 1:1 ethanol/freon, and 1:3 ethanol/freon; and twice in 100% freon. They were then placed in a critical point drier in CO₂. The tissues were mounted in silver paste and sputter-coated in gold palladium for 2.5 minutes.

Oocyst Counts

Fecal samples were collected from each litter every 1-2 days and from each individual pig at necropsy. Feces were mixed with water, passed through a wire cloth sieve and centrifuged (at approximately 1500 rpm for 5 minutes). Forty-five ml of water and 5 ml of ether were added to 2g of the washed sieved fecal sediment then mixed by inversion and centrifuged as

above. The lipid and supernatant layers were decanted and discarded. The sediment was diluted with Sheather's sugar solution, mixed thoroughly and put into McMaster chambers. The number of oocysts/gram of washed fecal sediment was calculated from the average count of four chambers. Samples negative by this technique were examined microscopically at 200x after centrifugal flotation (1500 rpm for 5 minutes) of the ether-treated washed fecal sediment in Sheather's sugar solution.

Evaluation for Other Gastrointestinal Pathogens

Fecal samples obtained from each litter at the onset of diarrhea and from each individual pig at the time of necropsy were evaluated for rotavirus by a commercial enzyme-linked immunoabsorbent assay (Rotazyme^R)¹. The sensitivity of this test to detect rotavirus infection within each litter was considered adequate because 10-15 samples/litter were tested. A section of ileum from each pig was examined for the presence of transmissible gastroenteritis virus by a direct fluorescent antibody method. Sections of duodenum, jejunum, and ileum were cultured aerobically on MacConkey's agar and blood agar plates in order to detect bacterial pathogens.

Statistical Analysis

The statistical model is summarized in Table 4. Most of the parameters fit the model with R^2 values averaging 0.71. The importance of the individual parts of the model were tested for each parameter by their F

1. Abbot Laboratories, Diagnostic Division; Chicago, Illinois

values and corresponding p values. (The level of p less than 0.05 was used to indicate significance). The statistics were used to determine the significance of individual numerical results using the least significant differences (LSD) at the 0.05 level.

Table 4: Statistical Model

Source	Degrees of freedom for the Villous Measurements	Degrees of freedom for the Crypt Measurements	Degrees of freedom for the Morpho- Metric Measurements
Model	123	153	76
Error (Error C)	320	512	94
A			
TRT ^a	1	1	1
Sow (TRT) (Error A)	6	6	4
B			
AGE ^b	4	4	4
TRT*AGE	4	4	4
SOW*AGE (TRT)	24	24	16
PIG(SOW*TRT*AGE) (Error B)	34	34	27
C			
LOC ^c	5 ^d	8 ^e	2 ^f
TRT*LOC	5	8	2
AGE*LOC	20	32	8
TRT*AGE*LOC	20	32	8

^aTRT = Control vs Infected Treatment Groups.

^bAGE = 2 days pi, 5 days pi, 9 days pi, 15 days pi and 25 days pi.

^cLOC = Location within the small intestine.

^dLOC = Duodenum, jejunum-100, jejunum-200, lower-jejunum, ileum-15, ileum-5.

^eLOC = Duodenum, jejunum-100, jejunum-200, lower-jejunum, ileum-15, ileum-5, cecum, proximal colon, spiral colon.

^fLOC = Duodenum, jejunum-200, ileum-15.

RESULTS

Clinical Signs

All control sows remained clinically healthy throughout the study. Control pigs remained clinically healthy as well. No Isospora suis oocysts were detected in their feces. Two of the infected litter sows became ill. One sow (999) suffered from mastitis two days post parturition. The udder was hot and hard, the milk production was decreased and watery, and the body temperature was elevated (T = 103.2). The sow was treated with oxytocin IM (10 IU), oxytetracyclines IM (5 mg/lb.) and udder hot packs twice a day for three days. The response to treatment was good. During these three days the piglets of litter 999 continued to suckle well. Supplemental milk was offered on the second day but little if any was consumed by the piglets.

Another infected litter sow (378) suffered from an elevated temperature (T=105.4) and hypogalactia for two days. The udder in this case was not inflamed and the milk did not appear grossly abnormal. The environmental temperature of the barns was elevated (95^o - 100^oF). This sow was treated conservatively with cold water baths and increased environmental air circulation. The piglets of litter 378 were offered supplemental milk (Soweena^R)¹.

Infected pigs began to vomit and to have diarrhea at 3 days pi. The time of onset was fairly consistent in all litters. Vomiting persisted for two days (days 3-5 pi). The diarrhea lasted 6-10 days (days 3-12 pi).

1. Merrick Inc.; Union Center, Wisconsin

At the first signs of digestive upset the pigs had tense tucked up abdomens and appeared uncomfortable and in abdominal pain. By the second day of vomiting and diarrhea many piglets began to look dehydrated and to develop rough to greasy hair coats. They seemed to cry more than the control piglets. The pigs were cranky and easily upset by stimuli. With the discontinuence of the vomiting, the worst of the clinical signs abated. By 6-8 days pi, even though the diarrhea continued, most piglets began eating more and behaving more sociably. The most severely affected piglets died between days 6 and 11 pi. The mortality rate of the infected pigs was 9.3% (Table 5).

Morphologic Lesions

Macroscopic lesions

No macroscopic lesions were found in the control pigs. Macroscopic lesions are described for each age group of infected pigs throughout the study.

Day 2 pi:

The pigs all had full stomachs. There were no lesions in the small intestine. The colon contents of the eight infected pigs ranged from a fluid yellow material to formed feces.

Day 5 pi:

Four of the eight infected pigs had empty stomachs. All eight pigs had thin, translucent intestinal walls and watery colon contents. One pig had moderately enlarged mesenteric lymph nodes.

Table 5: Mortality Rates

Group	Litter	Number of pigs Inoculated	Number of Deaths	Mortality Rate
Control	377	0	0	0%
	379	0	0	0%
	708	0	0	0%
	738	0	0	0%
	Total	0	0	0%
Infected	378	11	2	18.2%
	384	12	2	16.7%
	728	12	0	0%
	999	8	0	0%
	Total	43	4	9.3%

Day 9 pi:

All eight pigs had full stomachs. The intestinal walls were thin. Colon contents varied from watery (1/8) to a pasty consistency (4/8) to formed feces (3/8). Two pigs had moderately enlarged mesenteric lymph nodes.

Day 15 pi - Day 35 pi:

No gross lesions were observed.

Microscopic lesions

The baseline age-related histologic changes observed in the control animals are described below:

The number of goblet cells (Figures 1 and 2) tended to be consistent in all ages of control pigs and along all locations within the intestine of the control pigs although the duodenum and large intestine tended to have more goblet cells within their crypts. The numbers of intraepithelial lymphocytes (Figures 3, 4, 5 and 6) were highest in the duodenal sections and lowest in the ileum-15 sections at all ages. The intraepithelial neutrophils (Figures 7 and 8) were most numerous within the duodenal sections of all control piglets with a peak at 15 days pi. The lamina propria was consistent in the degree of neutrophil infiltration (Figures 9 and 10) although at 2 days pi there was an increase of neutrophils in both the duodenal and ileal sections. The number of eosinophils (Figures 11 and 12) tended to be fewer in the duodenal sections. There was a significant increase in eosinophil numbers in the small intestine at 15 days pi and 25 days pi in the lower-jejunum and ileal sections. The mitotic rate (Figures

13 and 14) was consistently lower in the duodenal and large intestinal sections when compared to the jejunal and ileal sections. The mitotic rate was high at 2 days pi. From 5 days pi to 15 days pi the mitotic rate remained fairly constant. At 25 days pi the mitotic rate rose, especially in the jejunum-200 and lower-jejunum sections. The villi decreased in length over time (Figures 15 and 16). The villi were longest in the jejunum and shortest in the ileum (mean villous lengths of 28 pigs: duodenal villi = 848 um, jejunal villi = 1037 um and ileal villi = 688 um). The crypts increased in depth over time (Figure 17). The duodenal crypts were usually the deepest (mean crypt depths of 28 pigs: duodenal crypts = 228 um, jejunal crypts = 215 um and ileal crypts = 204 um). The villus/crypt ratio decreased over time (Figure 18). The highest ratio was found in the jejunum (mean villous/crypt ratios of 28 pigs: duodenal ratio = 2.928, jejunal ratio = 3.055, ileal ratio = 2.683).

Microscopic lesions of the small intestine for each age group of infected pigs are described below:

Day 2 pi:

There was little evidence of villous atrophy (Figures 15 and 16), focal necrosis (Figure 19), or villous fusion (Figure 20; Photograph 1A). Most villi and crypts were covered by a columnar epithelium. Of eight piglets, five had mild cuboidal changes at the villous tips within the duodenum and jejunum-100 sections (Photograph 2). Pigs from L378 had marked villous atrophy in the duodenal section (Photograph 2). Multinucleated epithelial cells were noted in sections of the upper small

intestine in 3/8 piglets (Photograph 3). A mild decrease in numbers of villous goblet cells was observed (Figure 1). An increase in villous intraepithelial neutrophils was noted throughout the small intestines (Figures 7 and 8) and was most prominent in the dome epithelium (Photograph 4A and B). Villous intraepithelial lymphocytes were increased within the duodenal sections of infected piglets (Figure 3 and Photograph 1A, B and C). One piglet had an area of moderate acute focal ulceration within the jejunum 100. The lamina propria was multifocally infiltrated with accumulations of neutrophils and eosinophils. The number of neutrophils within the lamina propria was significantly increased in the duodenum and ileum-5 sections (Figure 9 and Photographs 4A, B and C); whereas none of the eosinophil counts within the lamina propria were significantly different from the control counts (Figures 11 and 12).

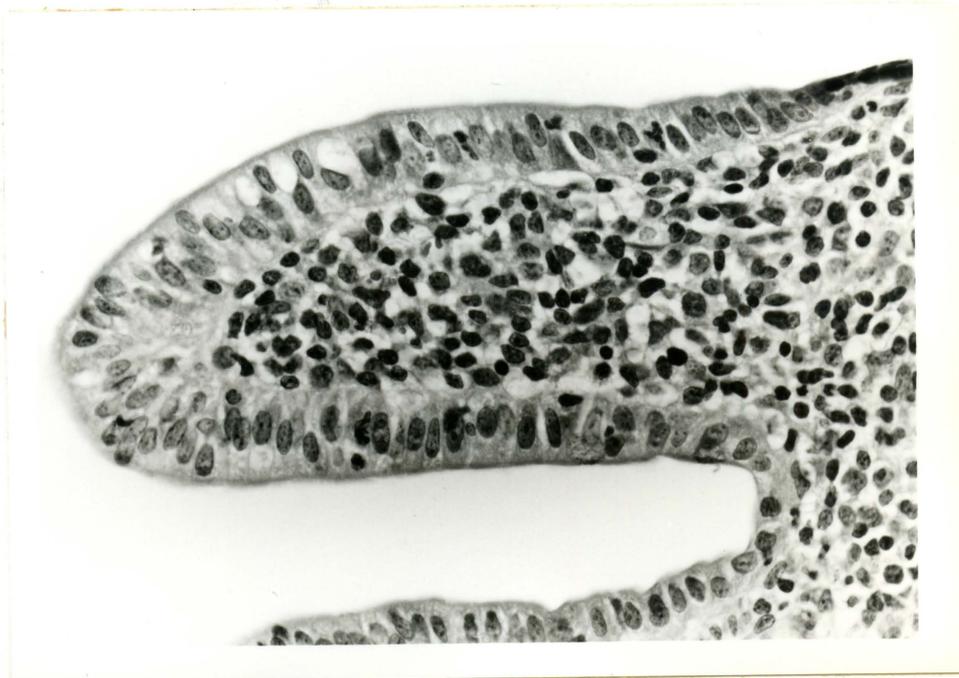
Parasites were seen in 5/8 piglets. A total of 24 parasites were observed; 19 were in villous epithelium, 5 were within crypt epithelium. Two of eight pigs were observed with parasites infecting dome epithelium (Photograph 5). Most domes were seen in ileum 5 sections; all parasites in domes were in the ileum sections. The distribution of parasites (Figures 21, 22, 23 and 24) consisted of asexual merozoites within the epithelium of the duodenum (11), jejunum-100 (5), jejunum-200 (3), lower-jejunum (2), and ileum-5 (3). No parasites were seen in the large intestine.



A. Large numbers of lymphocytes are present. 200X
Photograph 1. Duodenum, 2 days pi, infected piglet (L384-2).

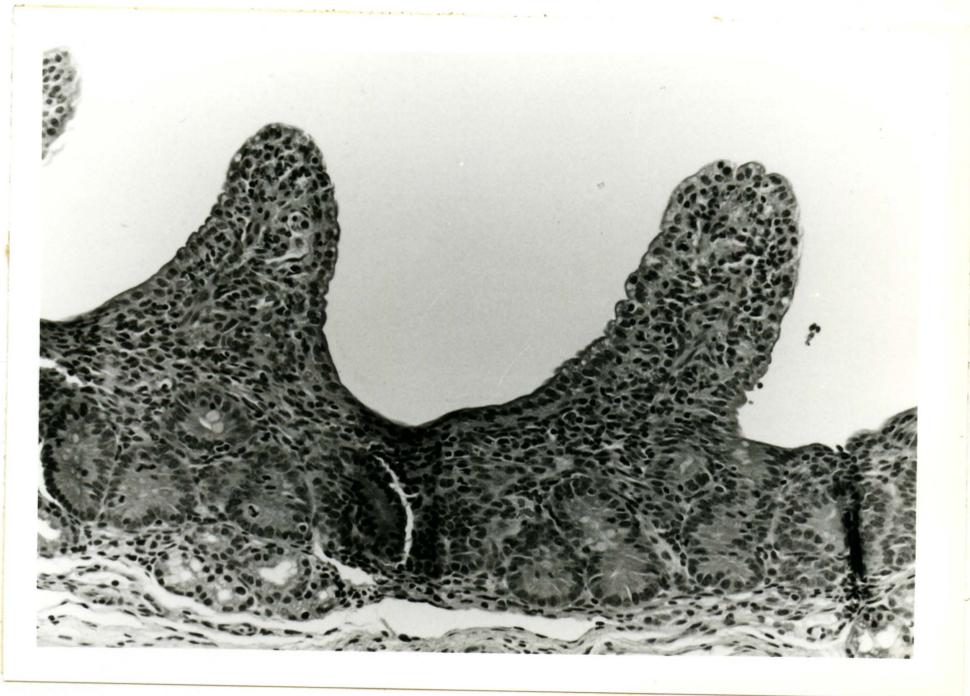


B. Numerous intraepithelial lymphocytes (arrow) occur. 500X



C. Many lymphocytes are in the lamina propria as well. 1250X

Photograph 1 (continued)



Photograph 2. Duodenum, 2 days pi, infected piglet (L378-1)

Epithelial changes (bulging, rounding, cuboidal cells), and numerous intraepithelial inflammatory cells are present. 500X



Photograph 3. Jejunum-100, 2 days pi, infected piglet (L384-2).

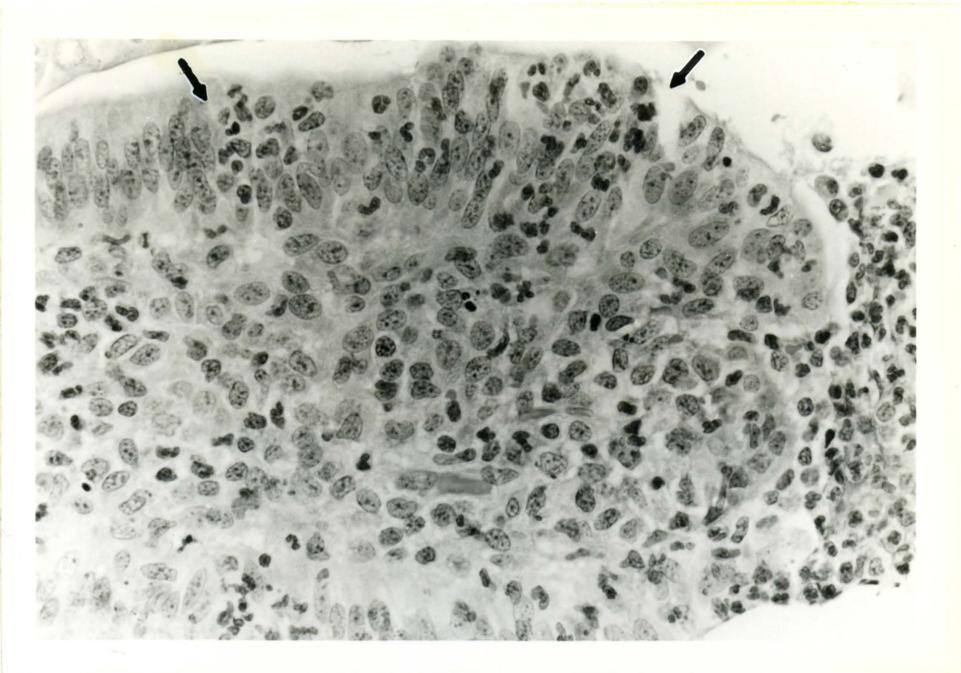
Cuboidal to squamous epithelial metaplasia and a large multinucleated epithelial cell (arrow). 1250X



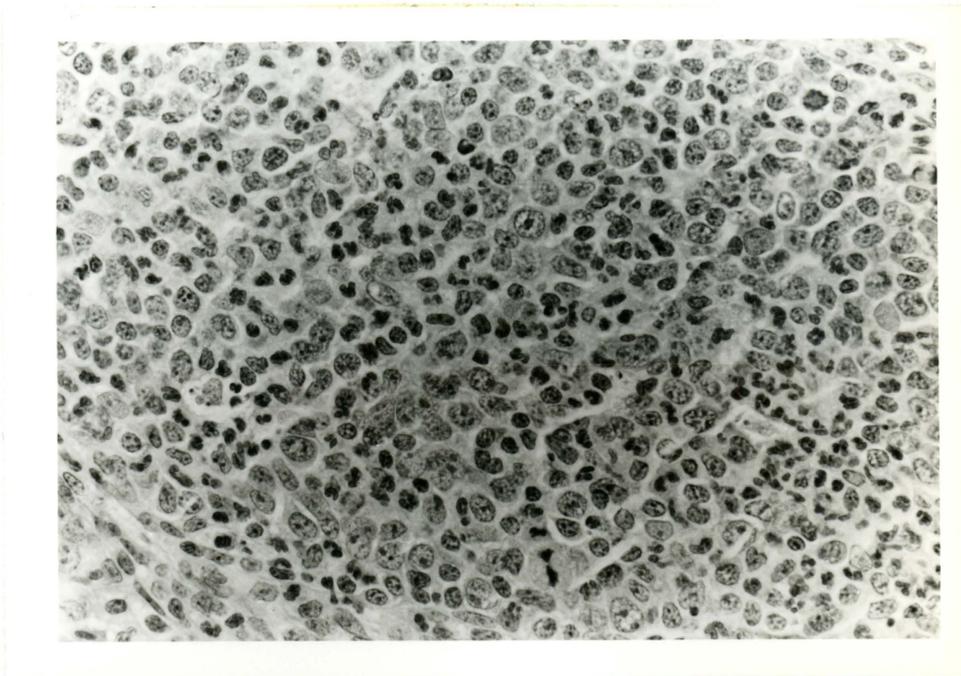
A. 200X

Photograph 4. Ileum-15, 2 days pi, infected piglet (L728-2).

A severe infiltration of neutrophils within both the epithelial cell layer and the lamina propria is present in the dome areas.

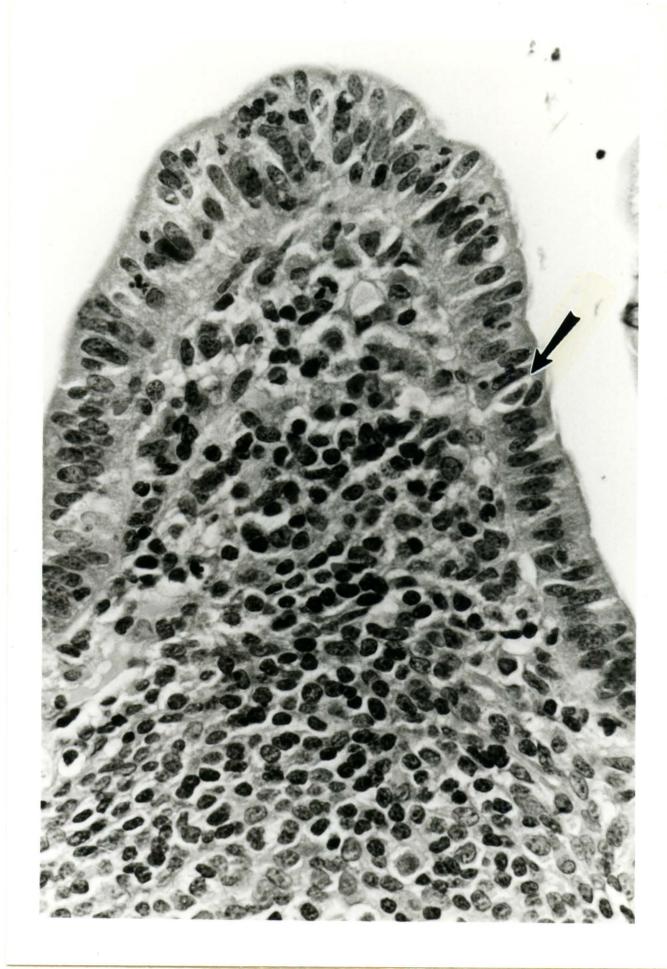


B. Epithelial infiltration (arrow) 1250X



C. Lamina propria infiltration 1250X

Photograph 4 (continued)

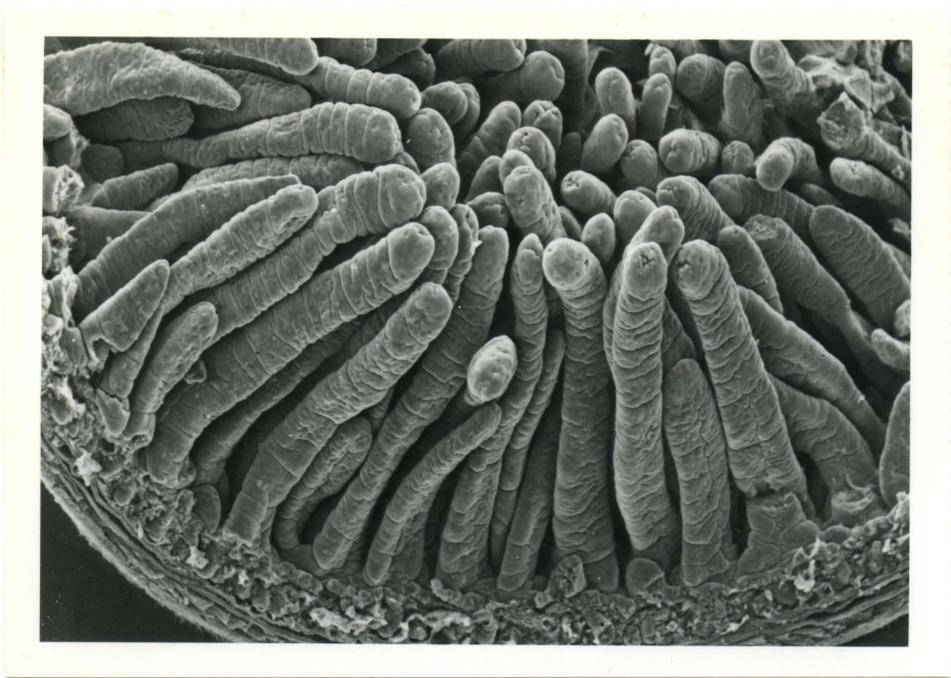


Photograph 5. Ileum-15, 2 days pi, infected piglet (L378-1)

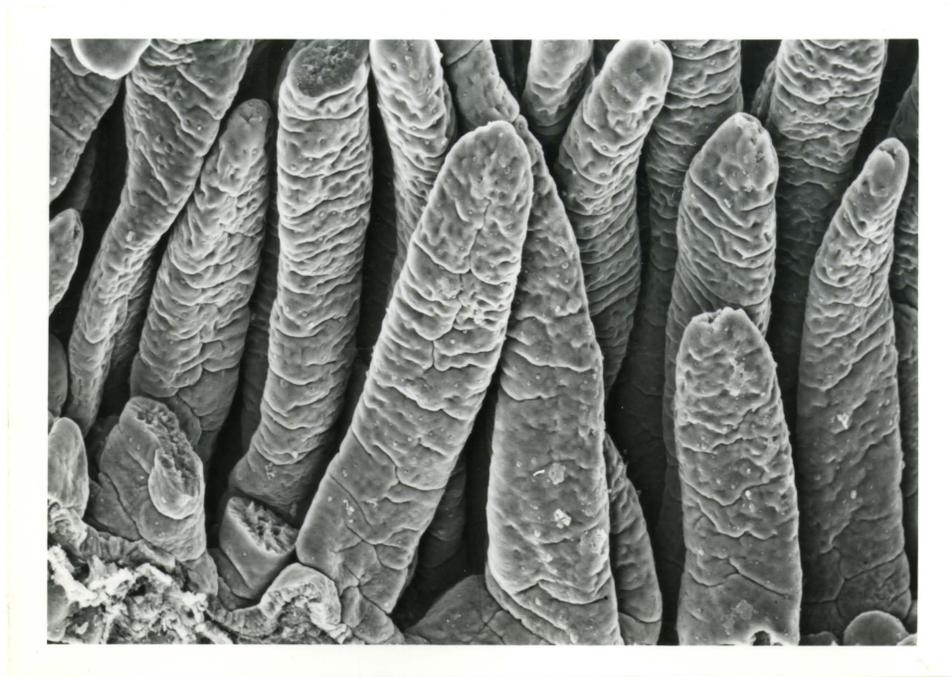
A merozoite (arrow) is present within an epithelial cell of a dome. Note the inflammatory cells within the lamina propria and the villous epithelium at the tip of the dome. 1250X

Day 5 pi:

Villous atrophy was noted throughout the small intestines and was most severe at the mid-jejunum where villi were short and blunt (Figures 15 and 16 and Photographs 6A, 7A, 8A, 9A, 10A and 11A). Villous fusion was prominent with a peak at jejunum-100 and jejunum-200 sections (Figure 20 and Photographs 7B, 9B and 11C). The epithelial cells on the villous tips appeared irregular, knobby and bulging as they degenerated and sloughed from their basement membrane (Photographs 7B, 7C, 9B, 9C, 9D and 10B). From the duodenum to the ileum, villi were covered with an increasing proportion of cuboidal epithelium. The most prominent cuboidal metaplasia occurred at the jejunum-200 and lower-jejunum. There was squamous metaplasia in the ileum in 6/8 piglets. Focal microulcers most numerous in the lower-jejunum sections were observed in all piglets. Disorganized multinucleated epithelial cells were noted in 6/8 piglets being most prominent in the jejunum-200 and lower-jejunum sections. Domes were covered with sloughing epithelial cells mixed with neutrophils. Very few goblet cells were present in the altered villi. Throughout the small intestine the number of villous intraepithelial neutrophils (Figures 7 and 8) was increased, most markedly in the mid-jejunum to ileum, whereas the number of villous intraepithelial lymphocytes (Figures 3 and 4) was reduced. In crypts there was an increase in the number of mitotic figures (Figures 13 and 14) and an increase in the number of intraepithelial lymphocytes (Figures 5 and 6). Five piglets had crypt abscesses in the jejunum-100 to the lower-jejunum sections. Compared to controls, three of

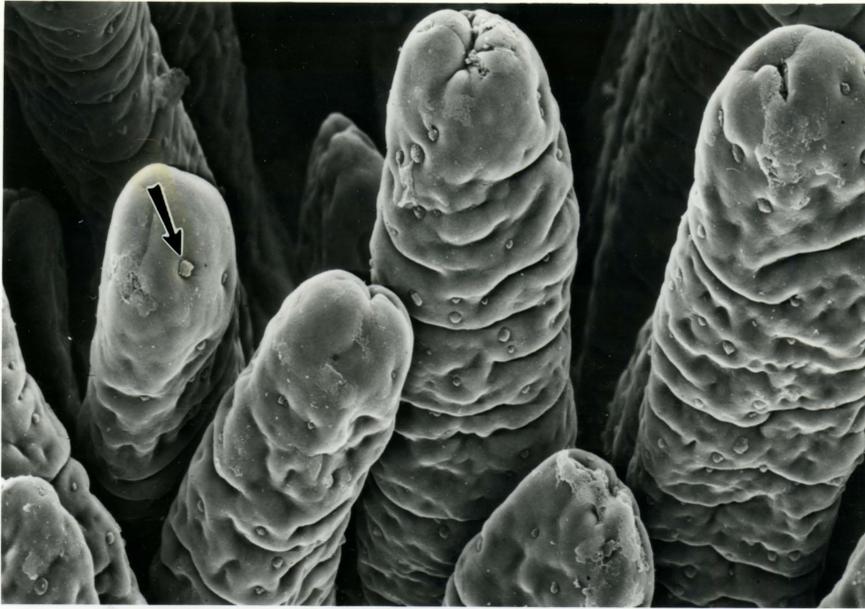


A. Note the tall slender smooth villi. 50.8X

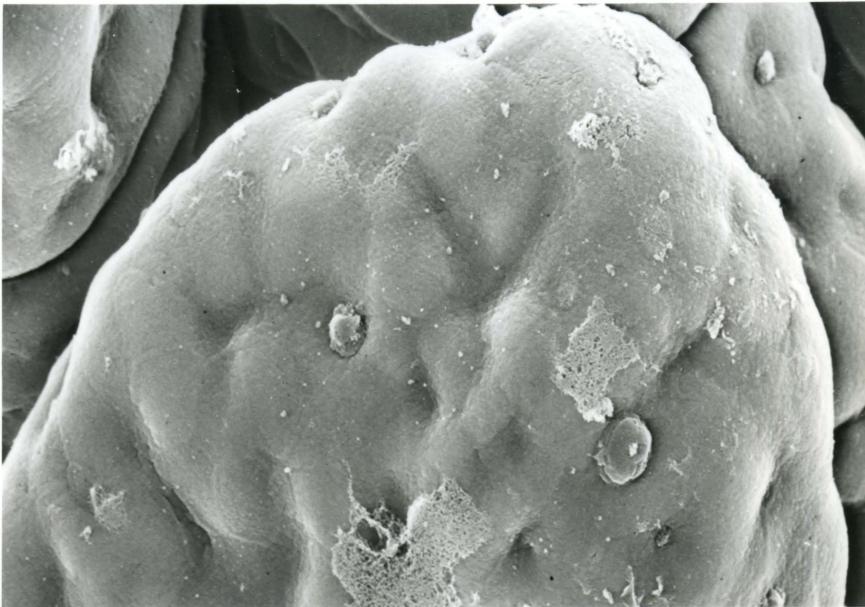


B. 101X

Photograph 6. Jejunum-200, 5 days pi, Control piglet (L708-3) scanning EM

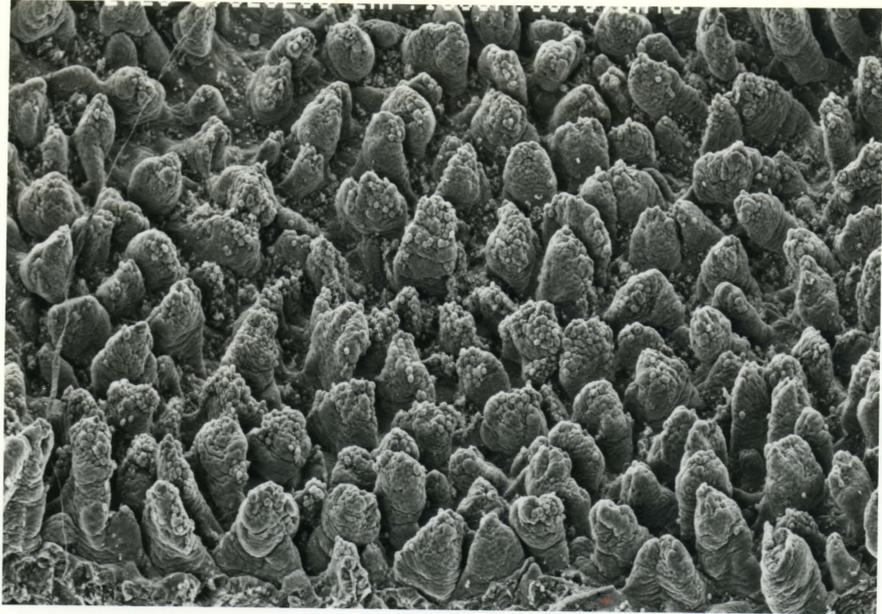


C. Note the goblet cells (arrow). 227X

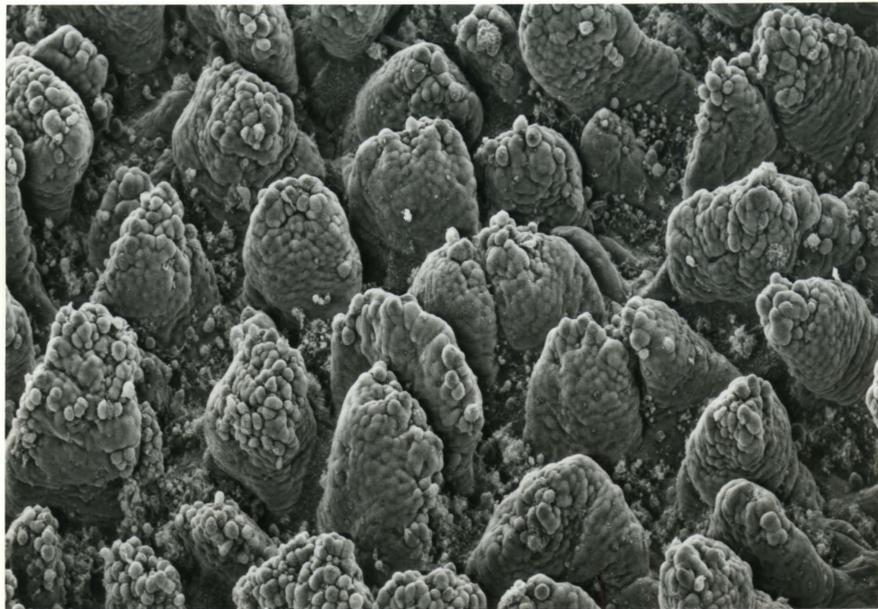


D. 886X

Photograph 6 (continued)

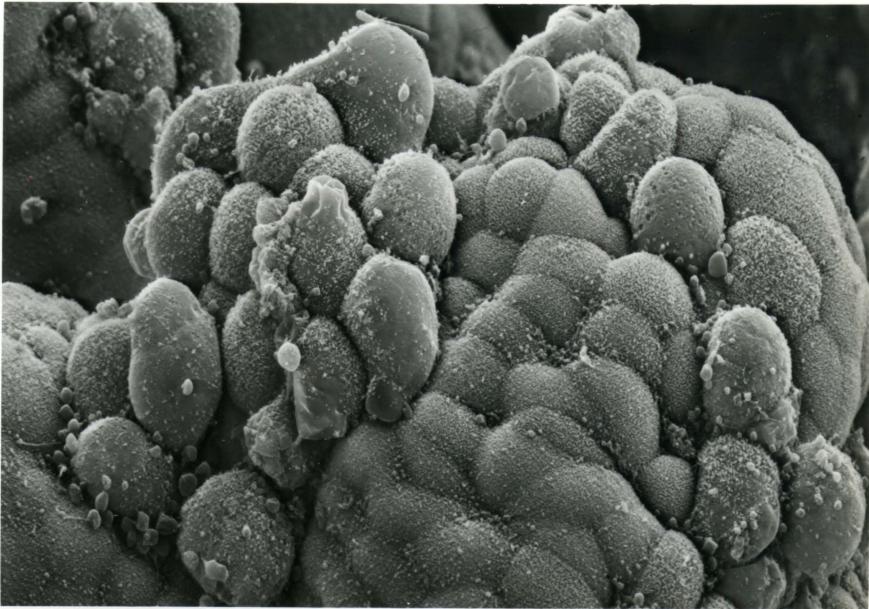


A. Note the villous destruction and atrophy. 49.1X



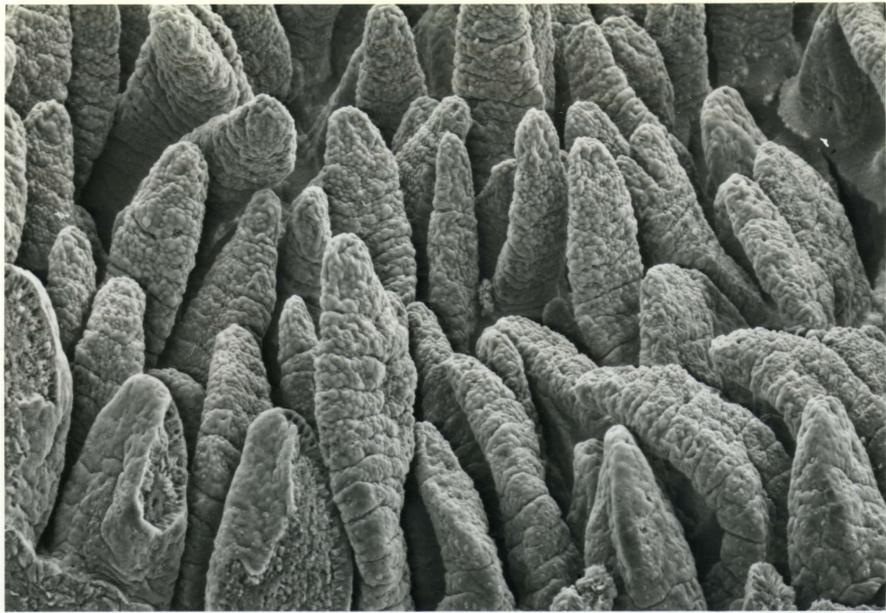
B. The epithelial cells round and bulge from the villi. Few goblet cells are seen. 107X

Photograph 7. Jejunum-200, 5 days pi, infected piglet (L728-3) scanning EM

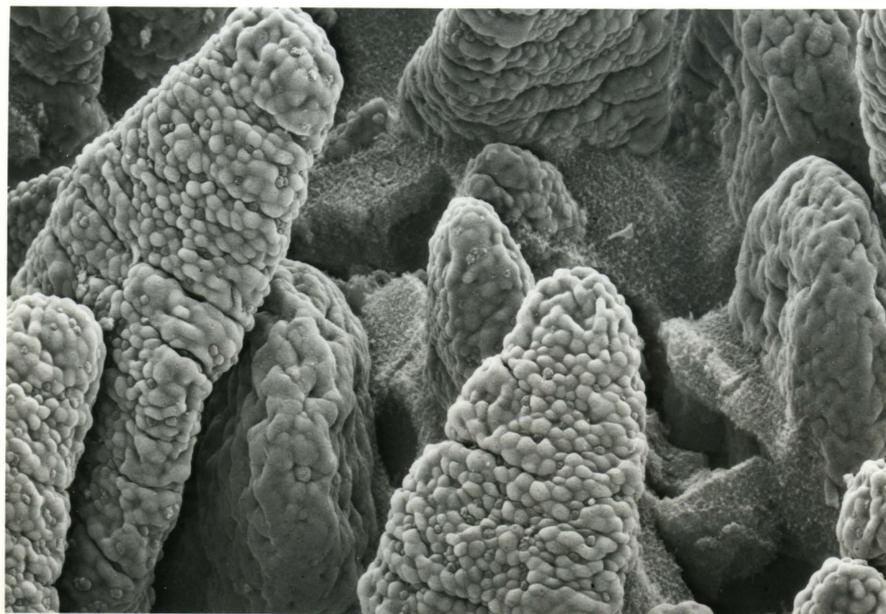


C. Necrotic epithelial cells are round and smooth with no microvilli.
822X

Photograph 7 (continued)

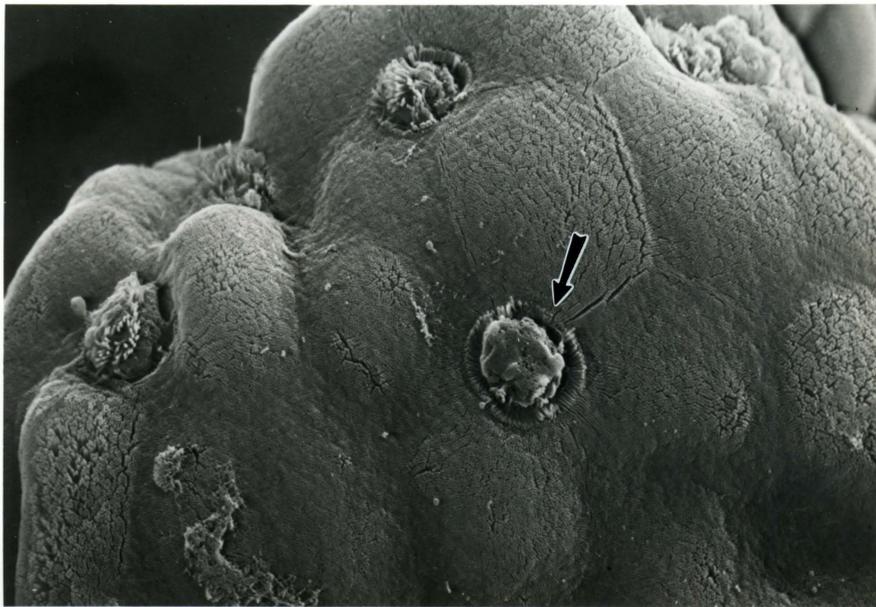


A. 76.4X



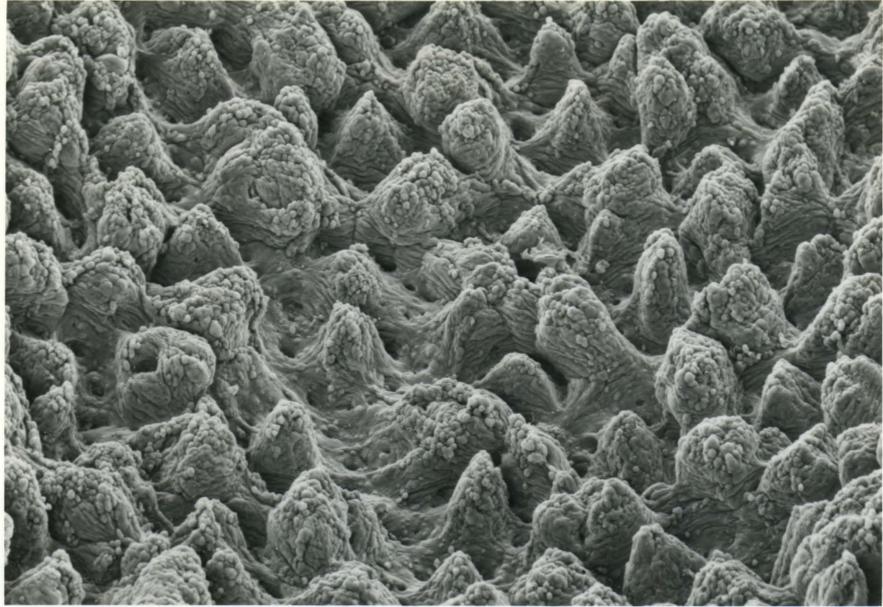
B. A few villi also have rounded bulging epithelial cells at the villous tips. 192X

Photograph 8. Ileum-15, 5 days pi, control piglet (L708-3), scanning EM

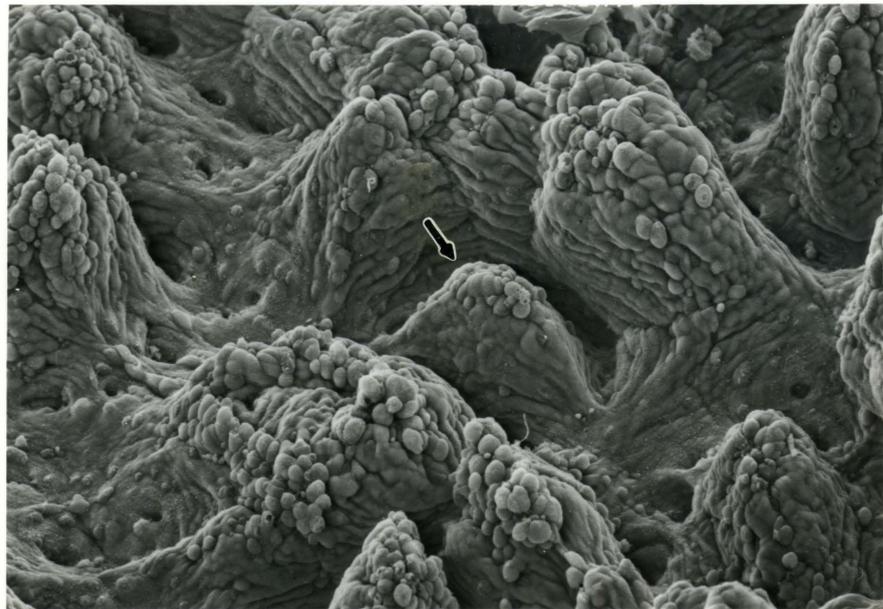


C. Note the smooth even cells covered with microvilli and the goblet cell (arrow). 2150X

Photograph 8 (continued)

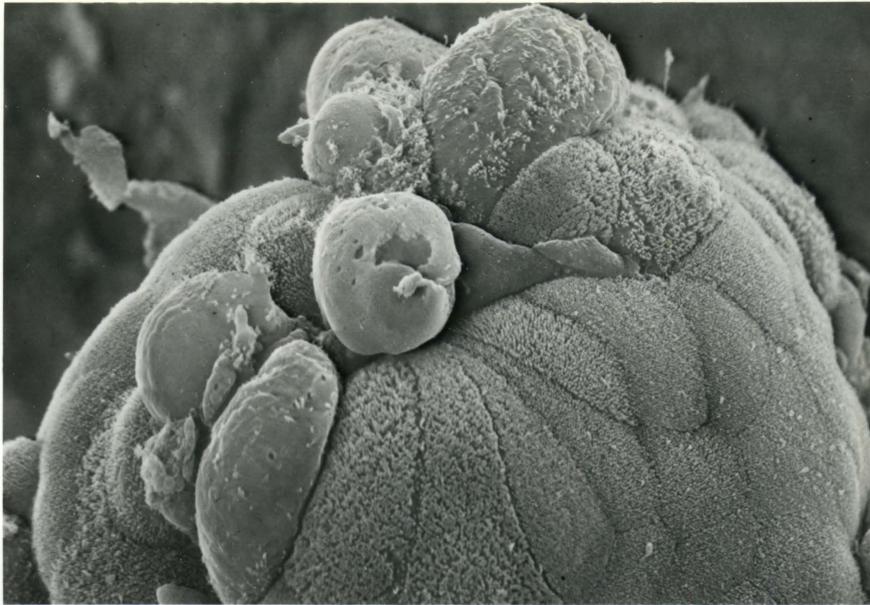


A. Note the villous destruction. 77.0X

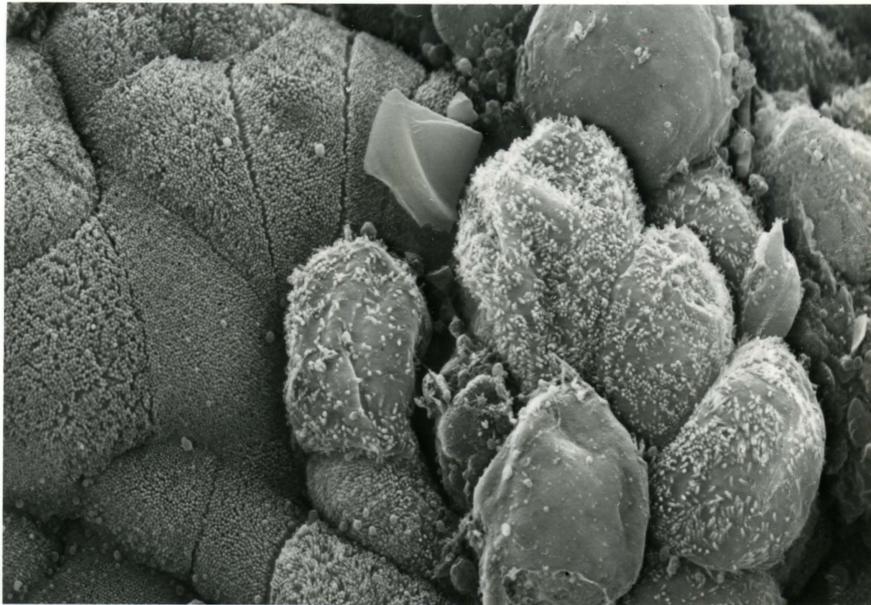


B. Villi fusion and villi with necrotic cells at their tips are shown. A presumed dome (arrow) with necrotic cells at its tip may be seen in the center. 197X

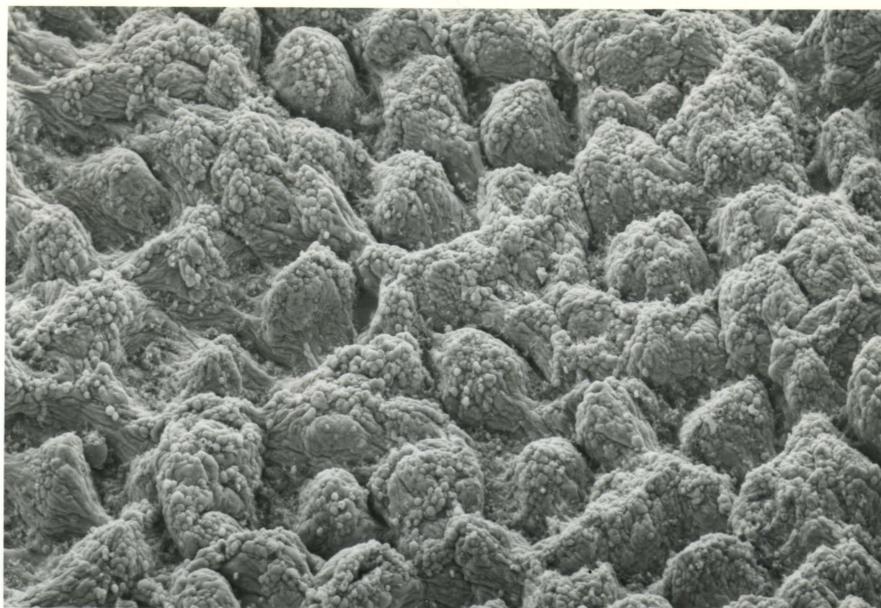
Photograph 9. Ileum-15, 5 days pi, Infected piglet (L728-3), scanning EM



C. Bulging, round, necrotic cells with no microvilli at the tips of a villus. 1720X



D. Degenerating, sloughed epithelial cells. 2080X

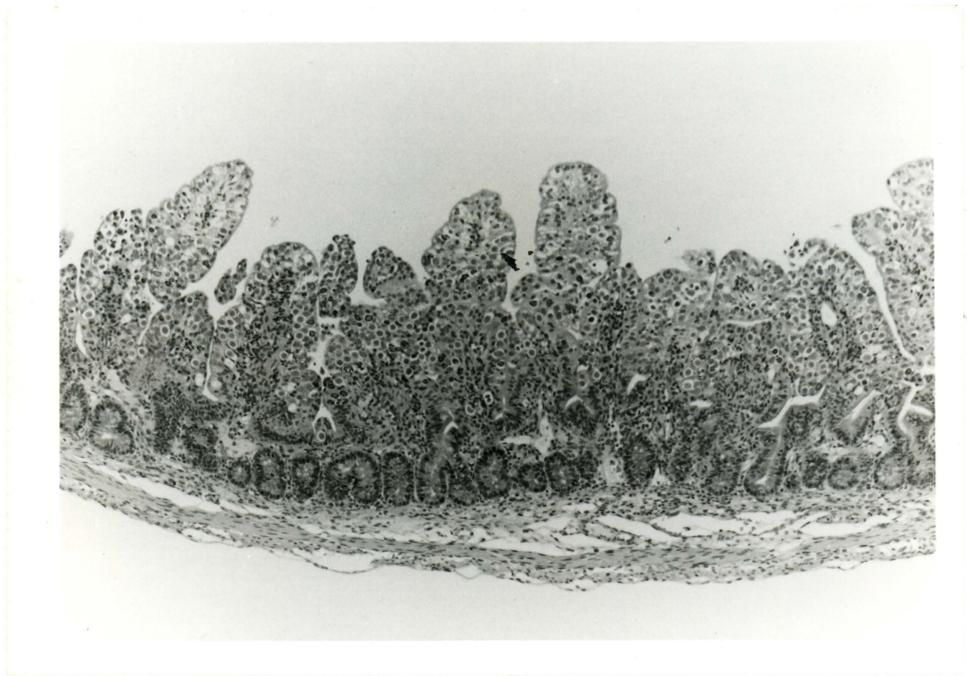


A. 77.6X



B. Note the smooth, round, bulging, necrotic cells at the tips of short villi. 204X

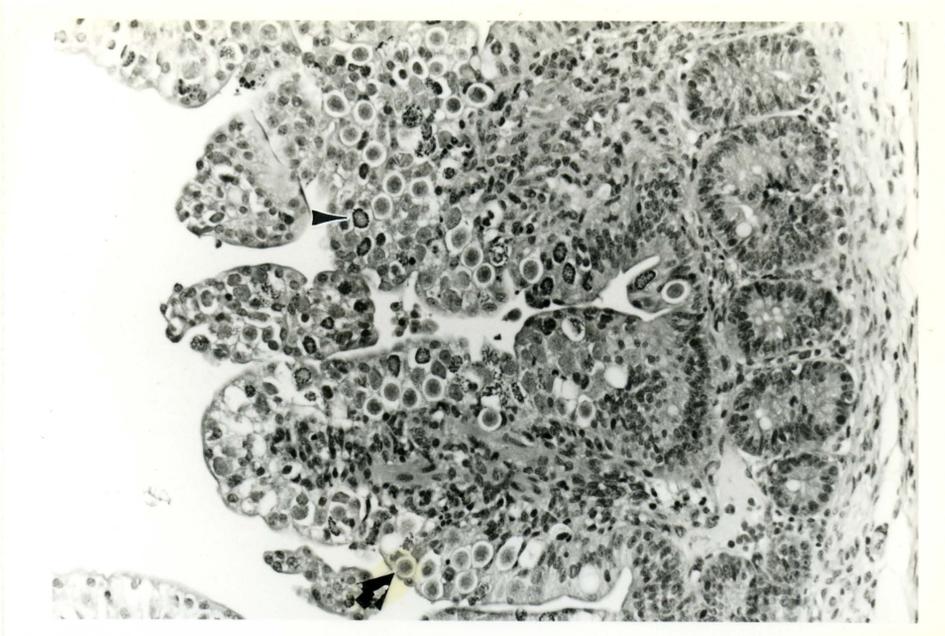
Photograph 10. Ileum-5, 5 days pi, Infected piglet (L728-3), scanning EM



A. Note the villous atrophy, the epithelial distortions and disorganization. 200X

Photograph 11. Jejunum-200, 5 days pi, infected piglet (L384-3)

In this study, the peak of Isospora suis parasitism appears at 5 days pi. All stages of the life cycle can be seen. The majority of epithelial cells are infected.



B. The most obvious and numerous stages seen are macrogametocytes (large arrow) and microgametocytes (small arrow). 500X



C. Early villous fusion may be due to cell surface changes caused by the invading parasites. 500X

Photograph 11 (continued)

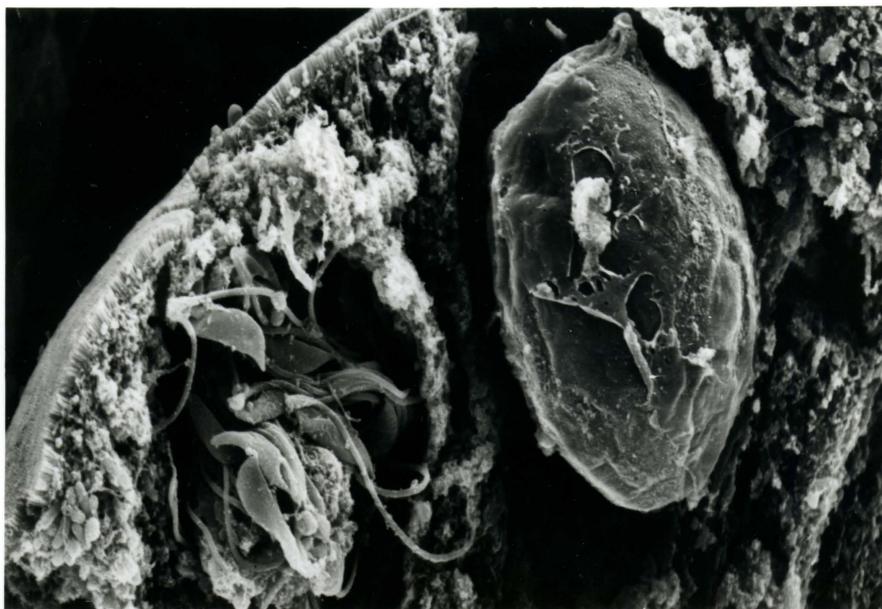


Photograph 12. Ileum-15, 5 days pi, Infected piglet (L999-4) scanning EM

Parasite stages are seen within parasitophorous vacuoles in epithelial cells. 806X

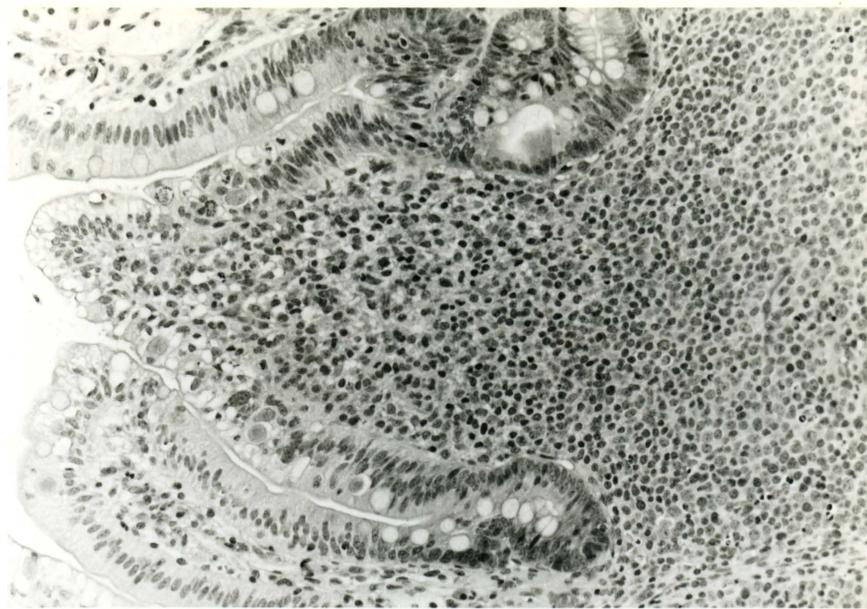


Photograph 13. Ileum-15, 5 days pi, infected piglet (L999-4) scanning EM
A packet of microgametes is present within an epithelial cell. 1770X



Photograph 14. Ileum-15, 5 days pi, Infected piglet (L999-4) scanning EM

Biflagellated microgametes and an oocyst are present within the epithelium of a villus. 2170X



A. Parasites (arrows) infect the dome epithelium. 500X



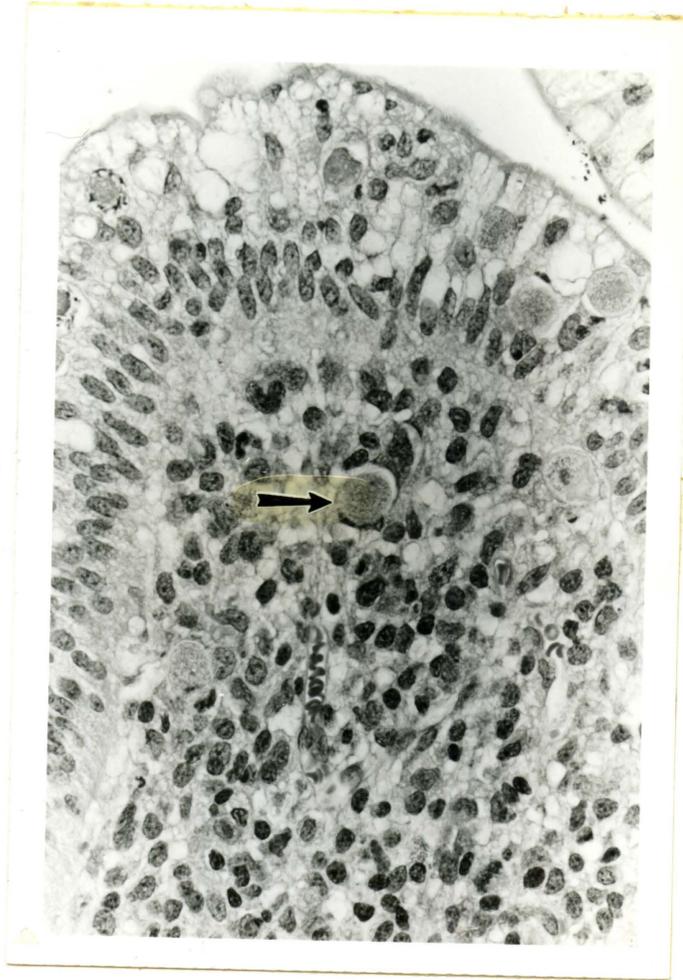
B. The large arrow identifies a macrogamete; the small arrow identifies a microgamete. 1250X

Photograph 15. Ileum-15, 5 days pi, infected piglet (L384-3)

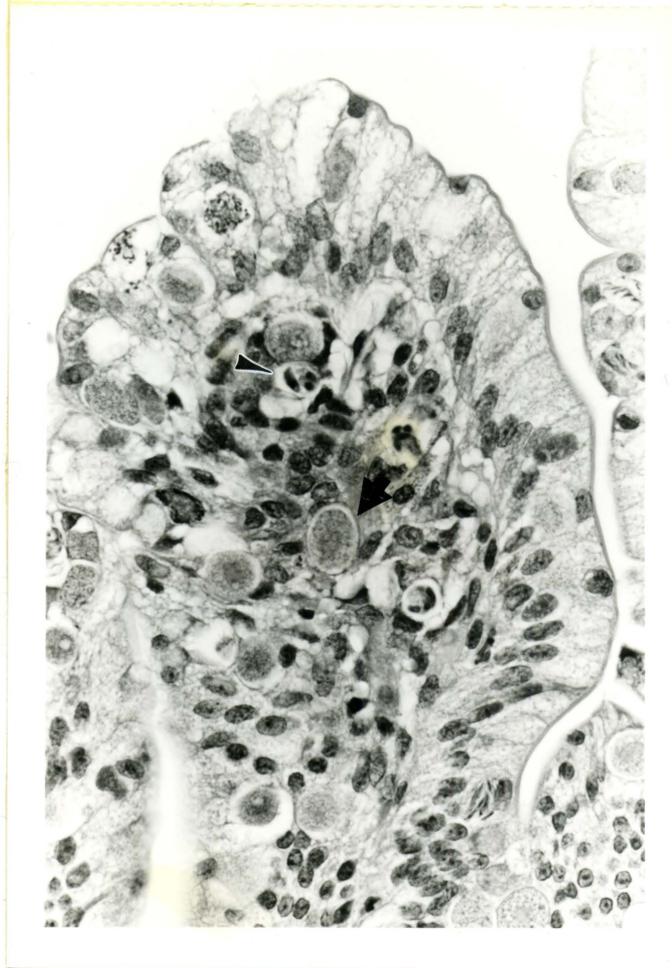


A. Parasites are present within the lamina propria and epithelium of domes. 500X

Photograph 16. Ileum-15, 5 days pi, infected piglet (L384-3)

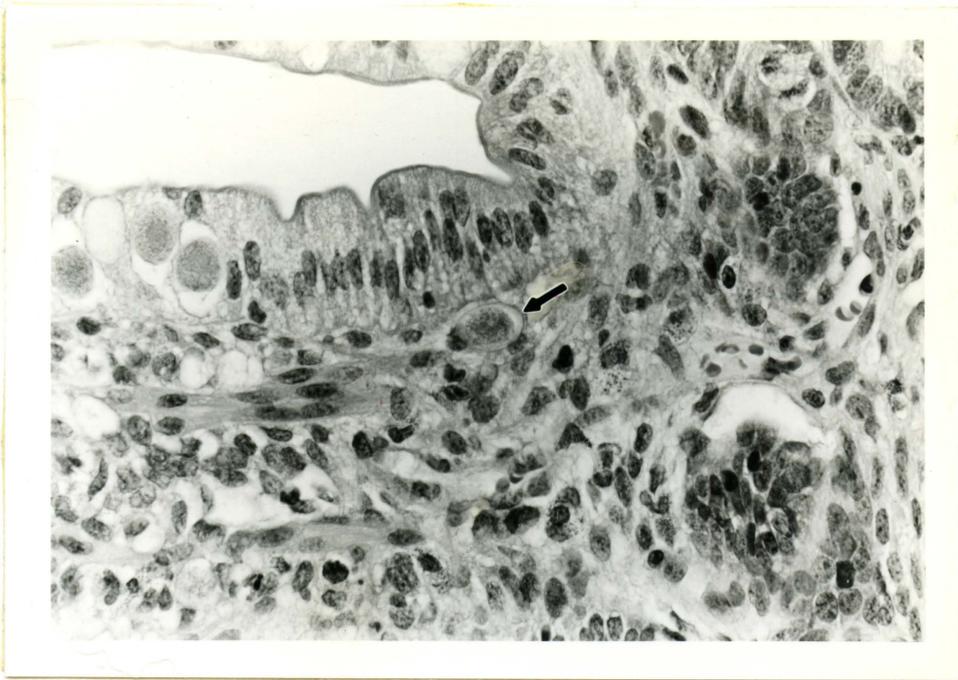


B. A parasite (arrow) within the lamina propria is indicated. 1250X
Photograph 16 (continued)

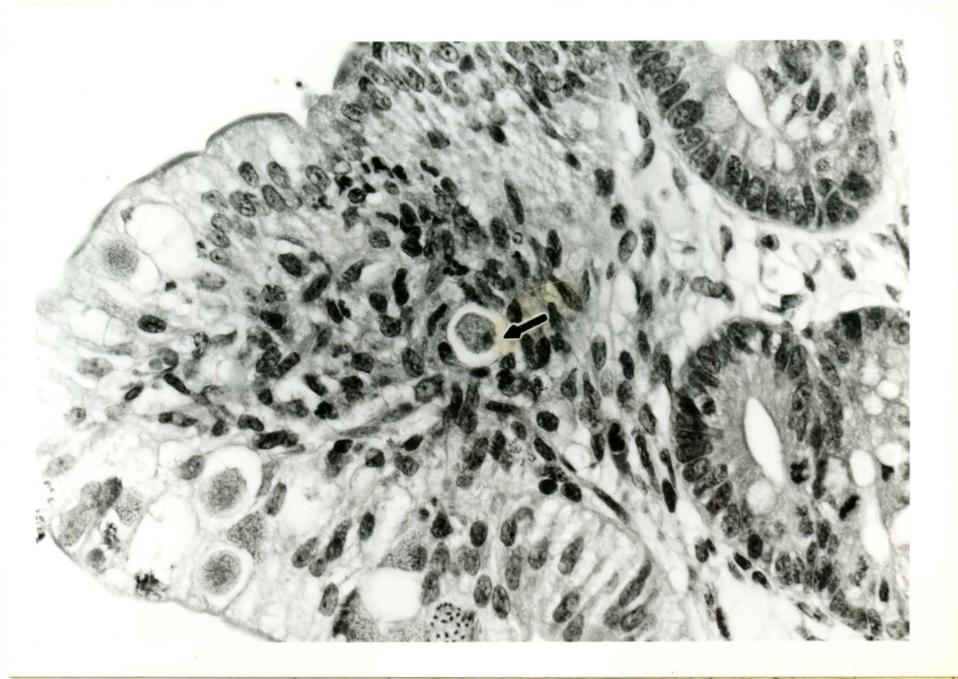


A. Parasites (large arrow points to a macrogamete; small arrow points to a merozoite) are present within the lamina propria of a villus. 1250X

Photograph 17. Jejunum-200, 5 days pi, infected piglet (L378-4)



B. A parasite (arrow) is beneath the epithelium. 1250X



C. Another parasite (arrow) within the lamina propria. 1250X

Photograph 17 (continued)

these piglets had fewer crosscuts of crypts at the lower-jejunum and ileum-15 sites.

Parasites were observed in all eight pigs at 5 days pi. A total of 23,445 parasites was seen. There was a large variation between litters (Figures 25 and 26). All stages of parasites were seen (Figure 25 and Photographs 11A, B, C, D; 12; 13 and 14). The parasites were most numerous at jejunum-200 to ileum-5 sections with the distribution recorded in Figure 27. Ninety-nine point sixty-two percent of these parasites were seen within villous epithelium. The remaining 0.38% were within crypt epithelium. Parasites within crypts were distributed as they were in the villi (Figure 23). Seven of eight pigs were observed with parasites infecting dome epithelium within sections of jejunum-200, ileum-15, and ileum-5 (Photographs 12A and B). A total of 479 parasites was counted in dome epithelium with the same stage distribution as seen in the villi. A number of parasites in several piglets were seen subepithelially within the lamina propria, primarily associated with domes (Photographs 13A, B, C, D and E).

Day 9 pi:

Six infected pigs from three litters (L378, L384 and L999) all had similar lesions. There were marked villous atrophy (Figures 15 and 16 and Photographs 14A, 15A, 16 and 17) and villous fusion (Figure 20 and Photographs 14B, 15A, 16 and 17). Many villi were covered with columnar epithelium with cuboidal changes present primarily at the tips of the villi. The cuboidal changes were most prominent in the lower-jejunum

section as was the degree of focal necrosis. The cells at the tips of villi were irregular, degenerate and sloughed (Photographs 14B and C). Microulcers were seen in five pigs, primarily around the lower-jejunum to ileum-5 sections. Disorganized, piled, multinucleated epithelial cells were often seen from the jejunum-200 to the ileum-15 sections. In many domes there was multifocal necrosis of the epithelium, with squamous metaplasia, a local infiltration of neutrophils, and fusion between domes and surrounding villi (Photographs 18A and B). There was a significant increase in numbers of intraepithelial neutrophils within villi, most prominent in the ileum-15 sections (Figures 7 and 8). There was a mild decrease of villous intraepithelial lymphocytes (Figures 3 and 4) as well as a decrease in the number of villous goblet cells (Figure 1). Crypt abscesses occurred in 6/8 pigs with a decrease in the number of crypt cross-sections in the lower-jejunum and ileum-15 sections occurring in 4/8 pigs (Photographs 19 and 20). Crypt hyperplasia was evident throughout the small intestinal sections. The mitotic rate was highest at 9 days pi (Figures 13 and 14) with counts varying from 3-5x the rate of the control pigs. Throughout the small intestine there was an increase in the number of crypt intraepithelial lymphocytes (Figures 5 and 6) as well as a mild increase in the number of crypt goblet cells (Figure 2). Parasites were observed in all eight pigs. There was a total of 126 parasites sited. All stages of parasites were represented (Figure 25). The parasite distribution peaked at jejunum-200 to lower-jejunum (Figure 27). Seventy point four percent of the parasites were in villous epithelium. Seven out



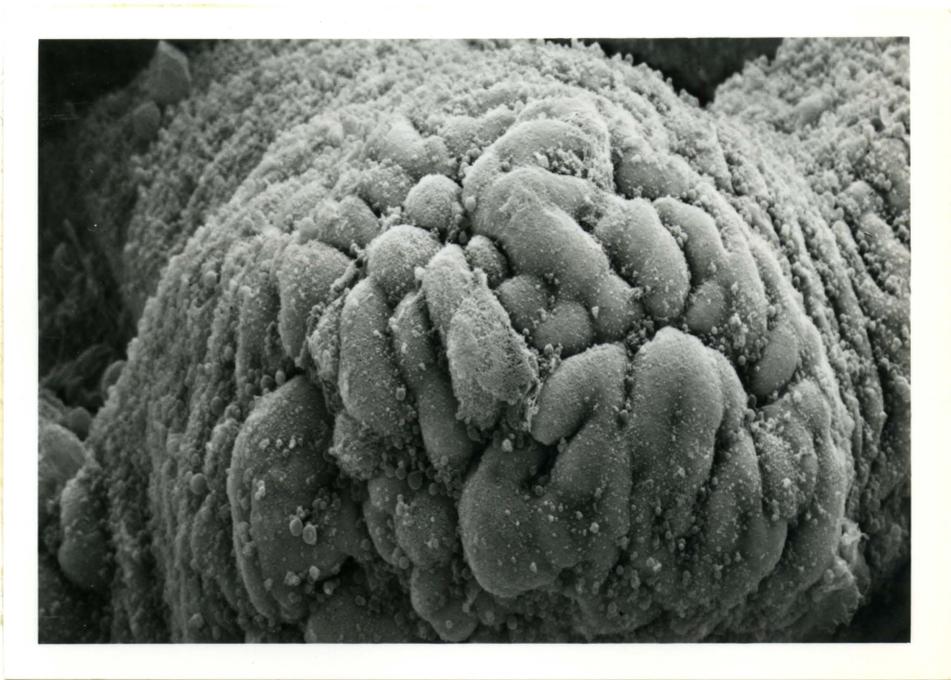
A. 77.2X



B. 202X

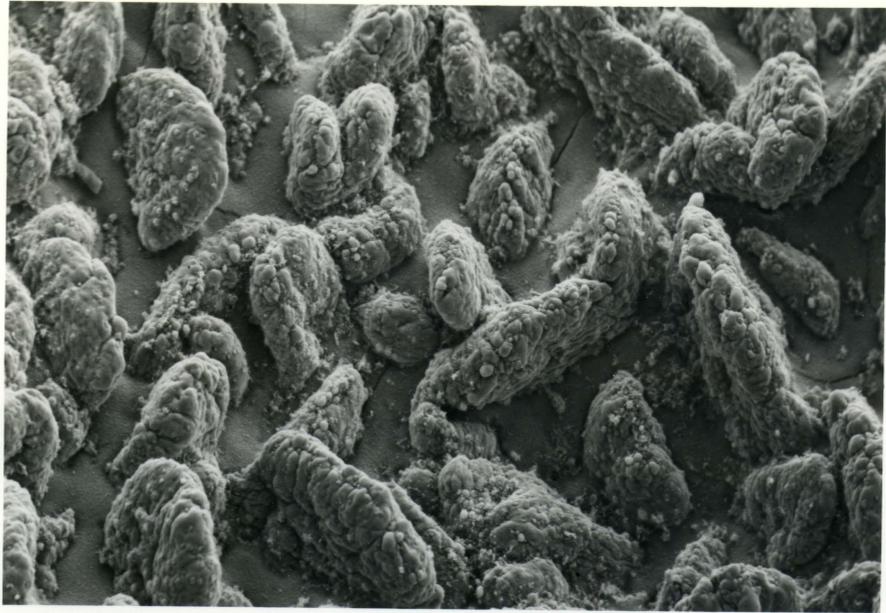
Photograph 18. Jejunum-200, 9 days pi, infected piglet (L728-6) scanning EM

The villi are short yet there are not as many necrotic, sloughed cells as there were at 5 days pi. Marked villous fusion is apparent.

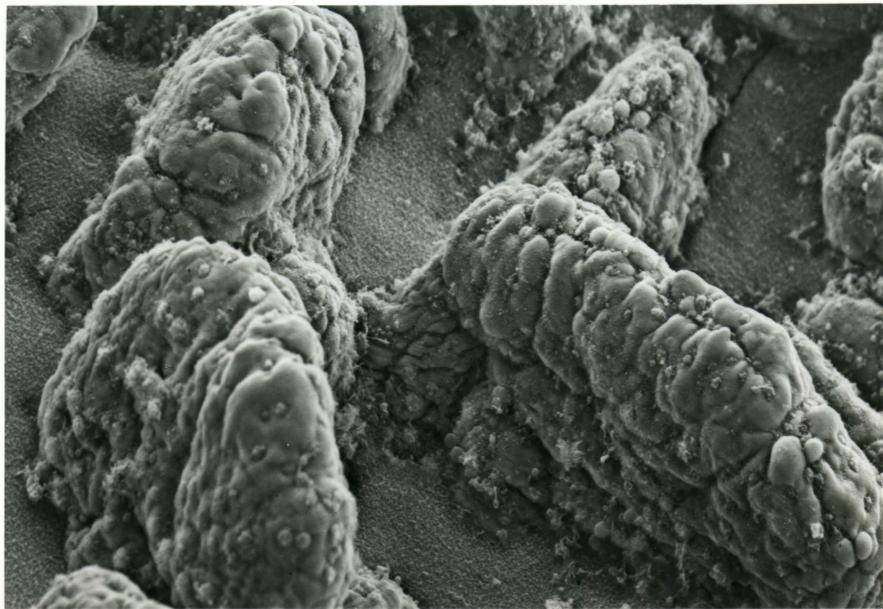


C. 808X

Photograph 18 (continued)



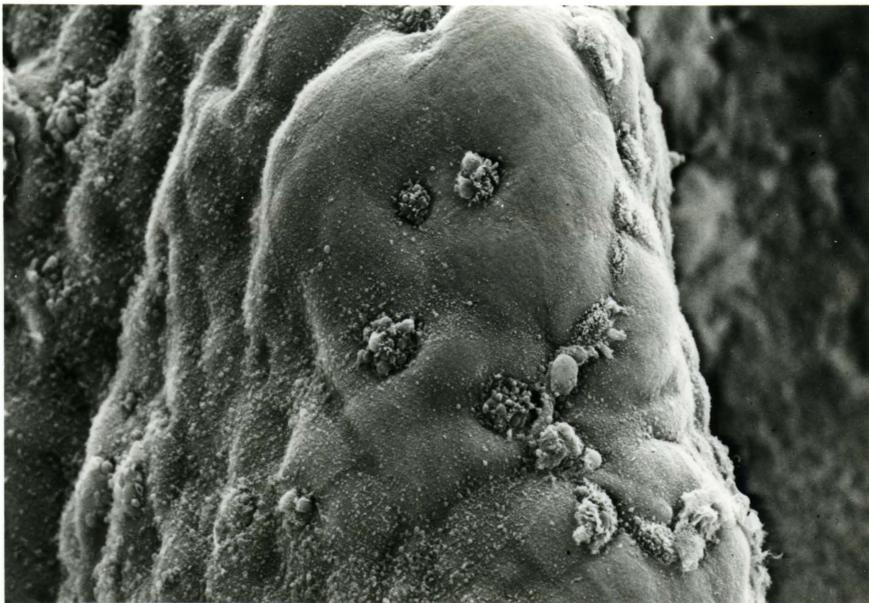
A. 77.5X



B. 203X

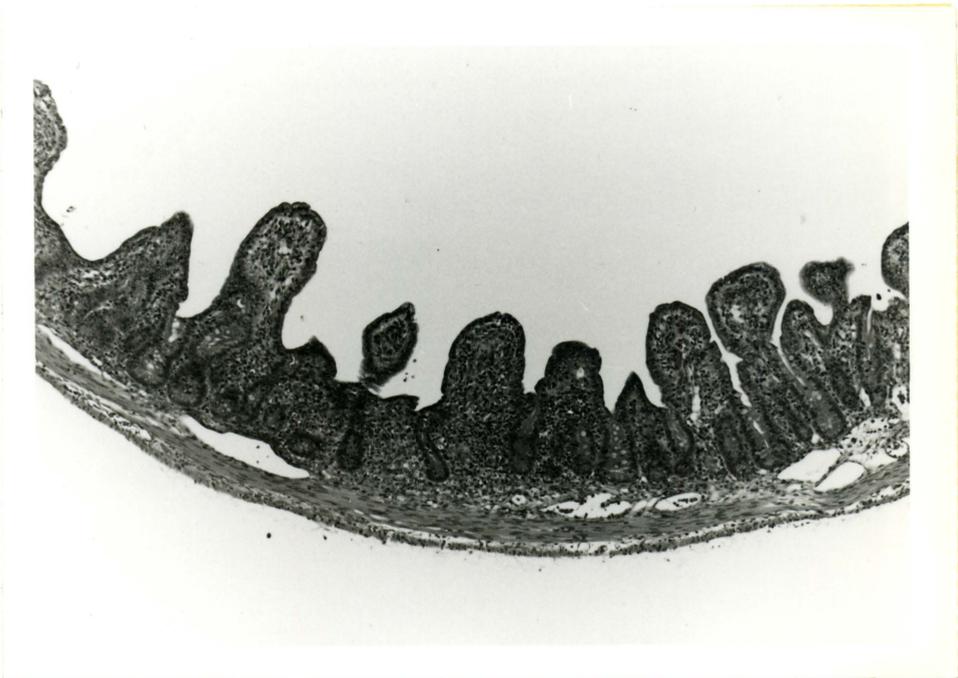
Photograph 19. Ileum-5, 9 days pi, infected piglet (L728-6), scanning EM

Villous atrophy and villous fusion are shown. Mucus fills the intervillus-crypt areas. There are not many necrotic epithelial cells. There are a number of goblet cells.



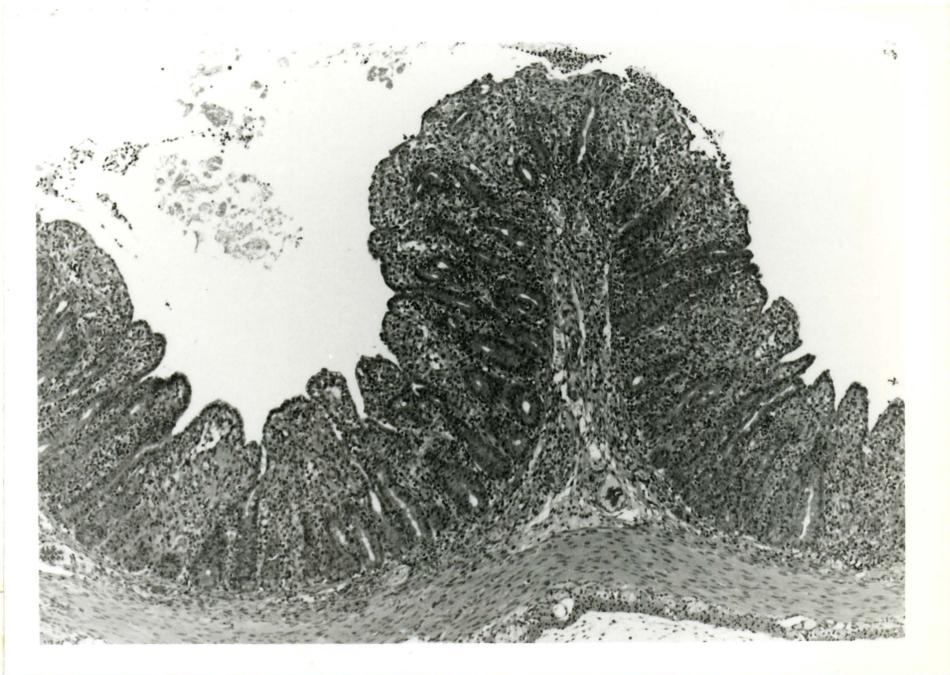
C. 818X

Photograph 19 (continued)



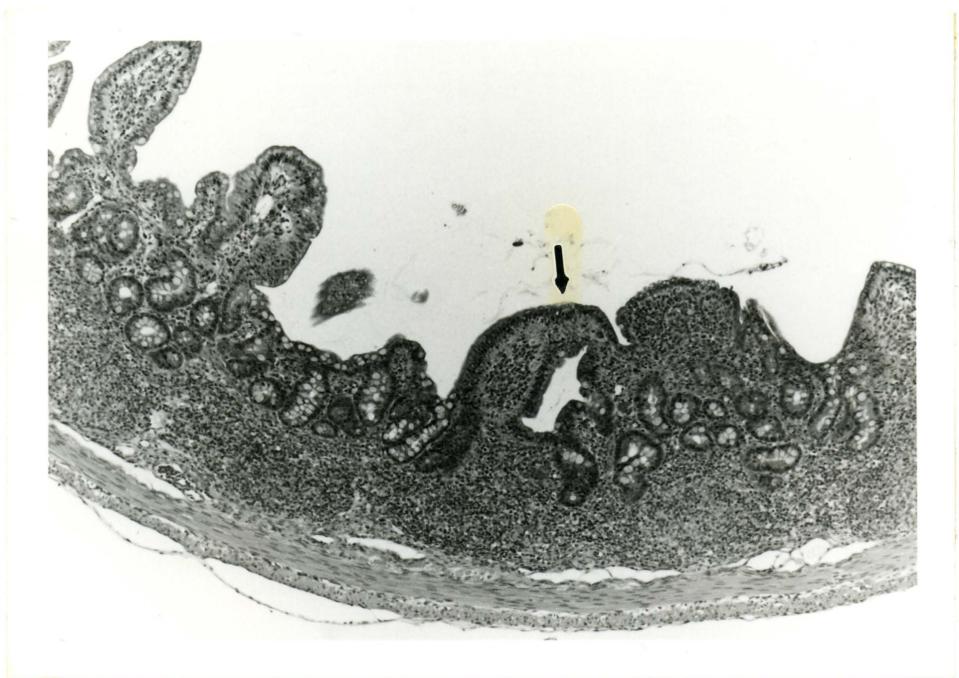
Photograph 20. Jejunum-200, 9 days pi, infected piglet (L378-5)

Villous atrophy is apparent. Villous epithelium is not fully recovered to columnar but is cuboidal, especially at the tips of the villi.
78.75X

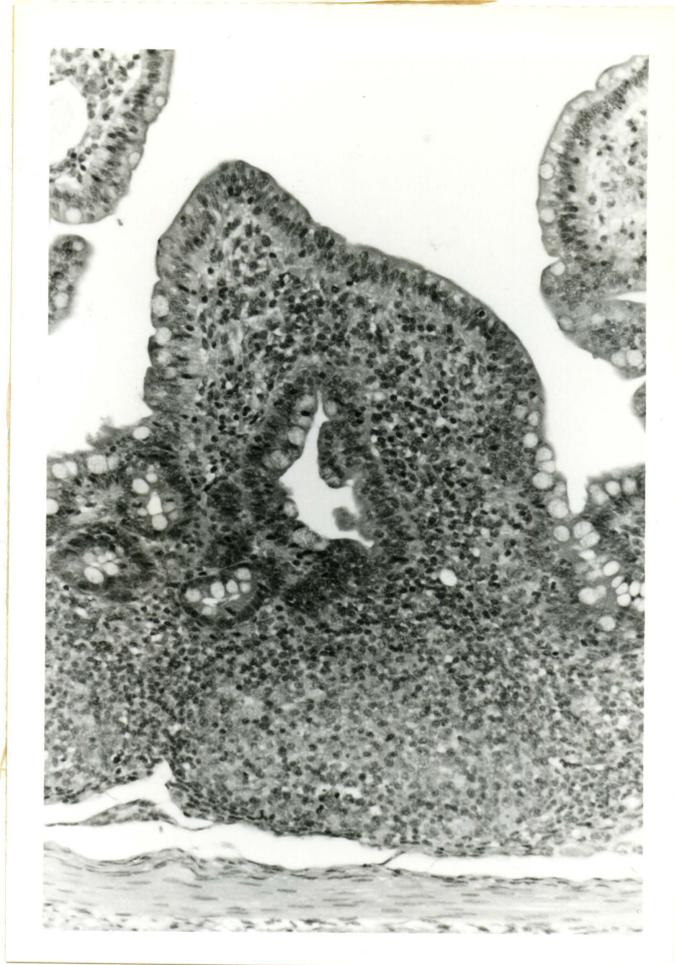


Photograph 21. Lower-Jejunum, 9 days pi, infected piglet (L378-6)

Villous atrophy and fusion predominate at 9 days pi. Epithelial changes and necrosis are still evident in localized areas. 200X

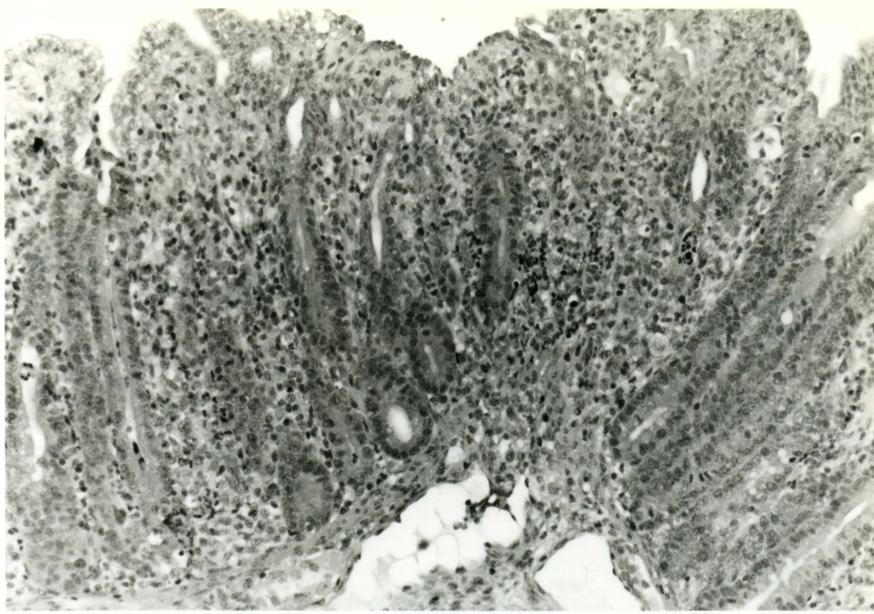


A. Villous fusion (arrow) and atrophy occur. 200X
Photograph 22. Ileum-5, 9 days pi, infected piglet (L378-5)

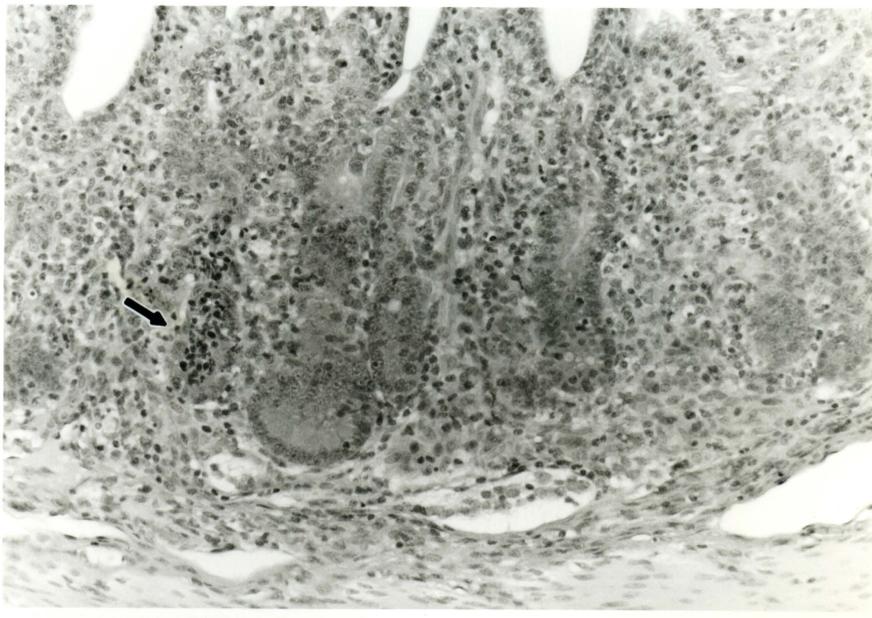


B. Fusion between domes. 500X

Photograph 22 (continued)



A. Crypt necrosis and a reduction in the number of crypts. 500X



B. Crypt necrosis with the formation of a crypt abscess (arrow).
500X

Photograph 23. Jejunum-200, 9 days pi, infected piglet (L378-6)

of eight pigs were observed with parasites in crypt epithelium. The crypt parasitic stages were similarly distributed as described above (Figure 23). One of the eight pigs was observed to have parasites within dome epithelium, the majority of which were asexual meronts (10/16 with 6 unidentified).

Two pigs from the fourth infected litter (L728) sacrificed at 9 days pi had less severe lesions. All of their villi were covered with a columnar epithelium. No focal necrosis or microulcerations were apparent. An occasional multinucleated epithelial cell was noted in the lower-jejunum through ileum-5 sections. Villous atrophy was mild (Figure 16). One of these pigs had a few crypt abscesses and an apparent decrease in the number of crypt cross-sections in the duodenum and jejunum-100 sections. Mild crypt hyperplasia with an increase in numbers of mitotic figures was observed. Although less heavily infected than the other infected piglets sacrificed at 9 days pi, both these pigs were parasitized with Isospora suis with asexual and sexual stages being sited in the crypts and villi of all sections of small intestine as well as in the proximal colon.

Day 15 pi:

Most epithelium was columnar but a few areas of upper small intestine still had mild cuboidal changes. Five of eight pigs had occasional multinucleated epithelial cells in the small intestines. There was a significant increase of villous intraepithelial neutrophils within the small intestine (Figures 7 and 8). Two pigs had an increase of neutrophil infiltration within domes and dome epithelium. There was a marked increase in numbers of villous intraepithelial lymphocytes (Figures 3 and 4). The



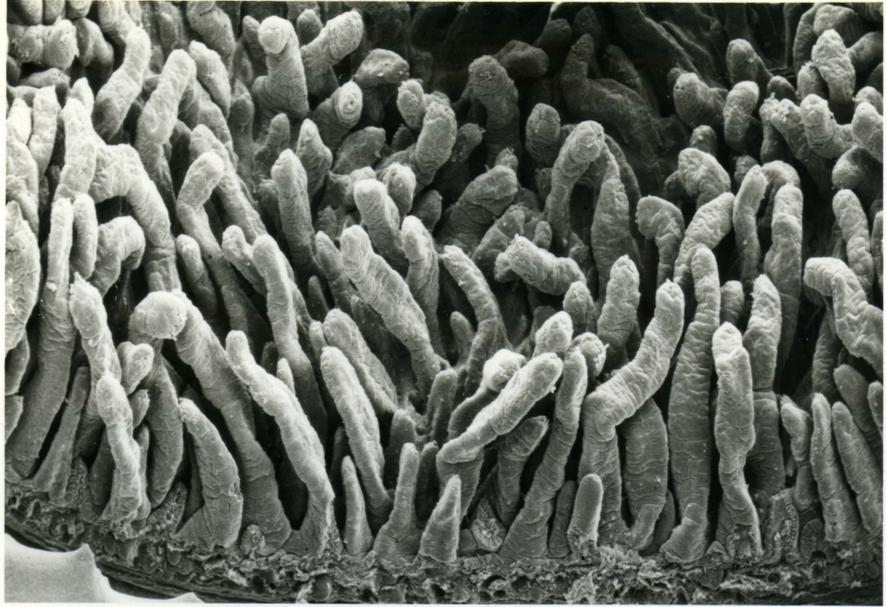
Photograph 24. Lower-Jejunum, 15 days pi, infected piglet (L384-7)

Recovering villi have regained both their length and columnar epithelium. Villous fusion is still present. 200X

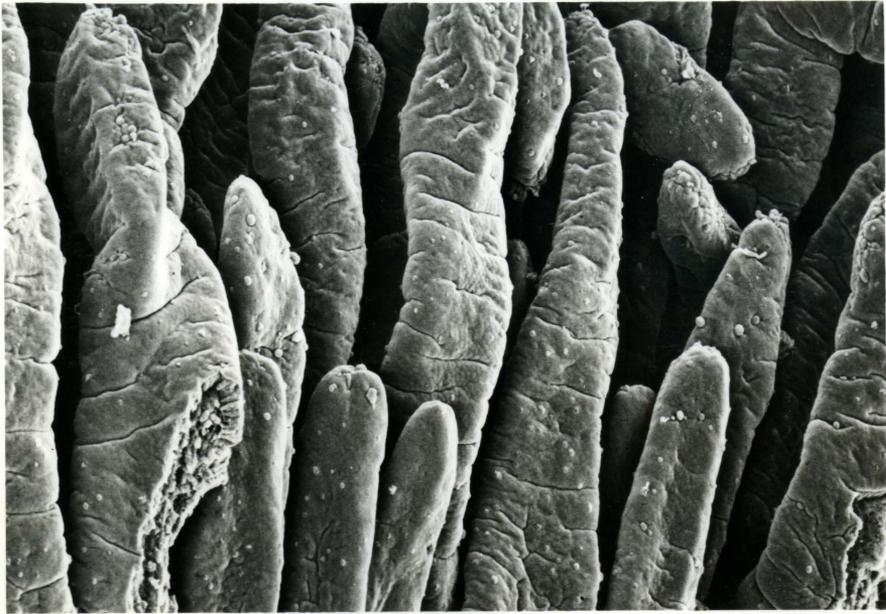
villi were longer than those found at 9 days pi and were covered by a columnar epithelium (Photograph 21). They appeared shorter and wider when compared to the controls (Figures 15 and 16). Obvious villous fusion was present (Figure 20 and Photograph 21). There was a slight reduction in goblet cell numbers within villi (Figure 1). The crypts were hyperplastic within the small intestine with mitotic rates well above control values (Figures 13 and 14). Five of eight pigs had crypt abscesses and two pigs had an apparent decrease in the number of crypt cross-sections in jejunum-200 and lower-jejunum sections. Three of seven pigs were observed to be parasitized. Parasites were sited primarily in crypts (20/27) and were primarily asexual meronts (19/27) (Figure 25). Parasites were present in all sections except ileum-5 (Figure 27). Ten of twenty-seven meronts were seen within the large intestine. Sixty-eight point two percent of the observed parasites were within crypt epithelium with only 31.8% in villous epithelium.

Day 25 pi:

All pigs had intestinal villi covered by columnar epithelium. The villi were comparable to controls as to height (Figure 15 and Photographs 22A and 23A). Mild villous fusion was observed (Figure 13 and Photographs 23B and 25A). No differences were noted between control and infected pigs in regard to either intraepithelial lymphocytes (Figures 3 and 4) or intraepithelial neutrophils (Figures 7 and 8). There was a very mild decrease in numbers of goblet cells within the small intestinal villi (Figure 1). The crypts had a mild decrease in mitotic figures when

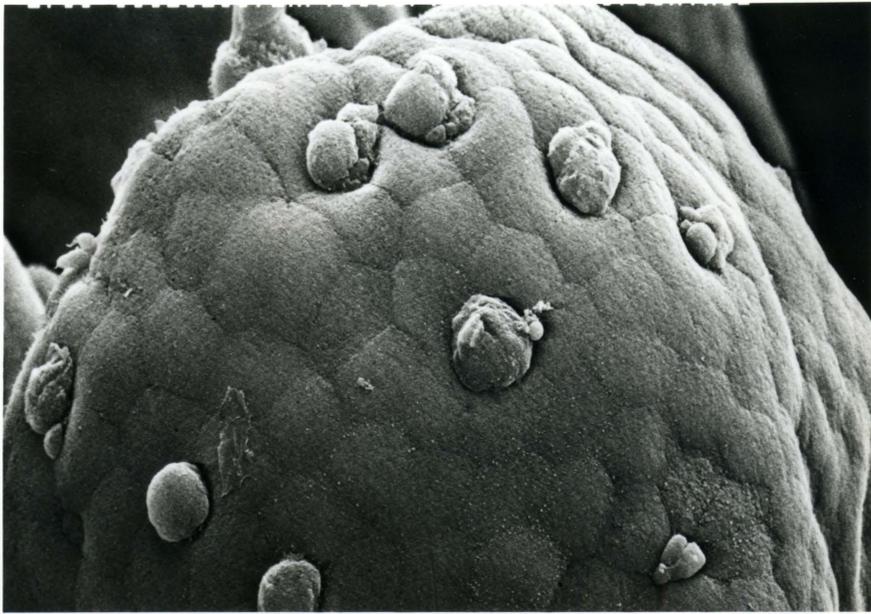


A. 34.2X



B. 95.2X

Photograph 25. Jejunum-200, 25 day pi, control piglet (L708-10), scanning EM

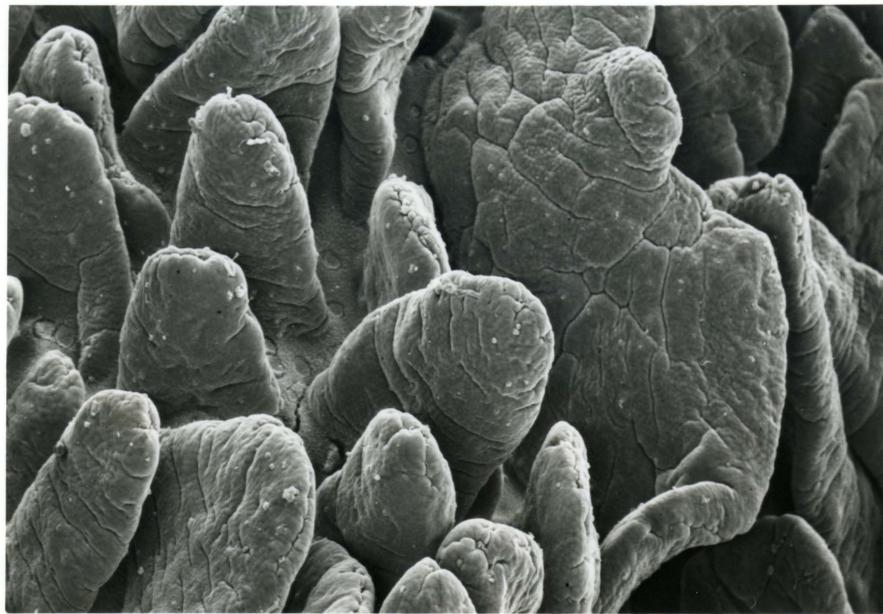


C. 1120X

Photograph 25 (continued)



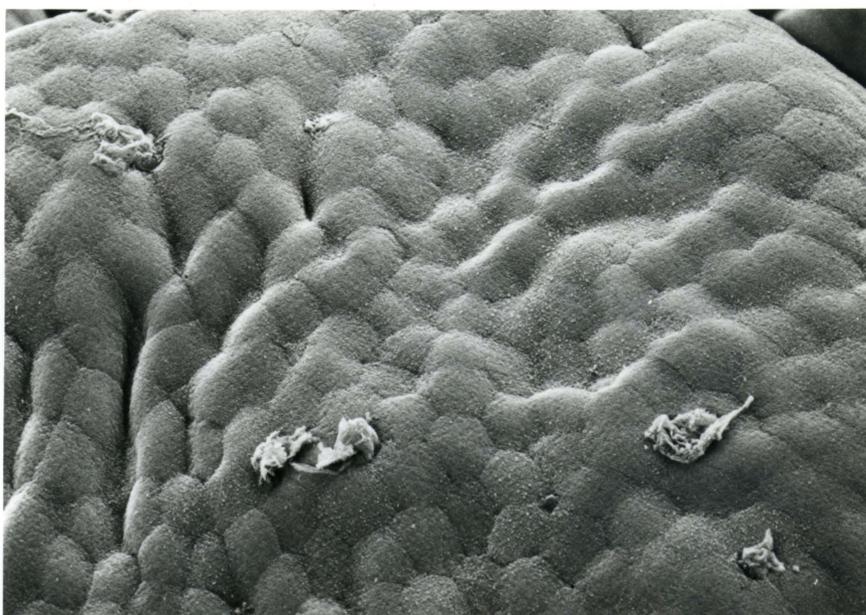
A. 32.9X



B. 94.2X

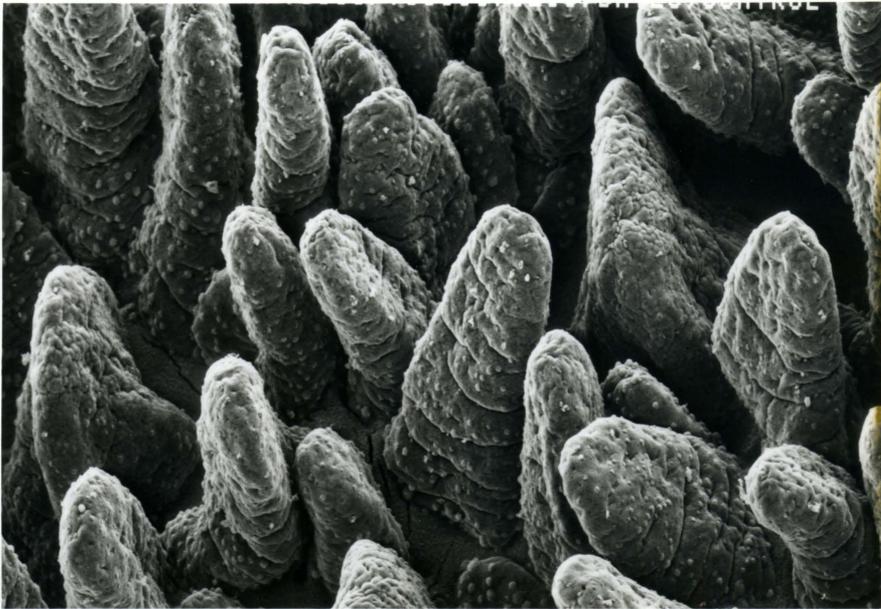
Photograph 26. Jejunum-200, 25 days pi, infected piglet (L384-9), scanning EM

Note the villous ridges presumably formed by villous fusion. Also note the villous recovery with smooth epithelial cells, and many goblet cells.



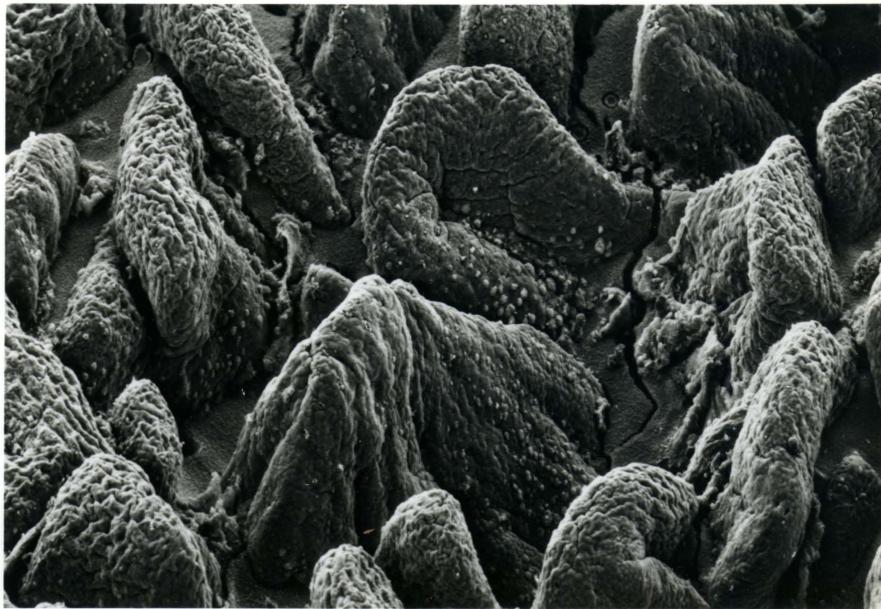
C. 1160X

Photograph 26 (continued)



Photograph 27. Ileum-15, 25 days pi, control piglet (L708-10), scanning EM 90.7X

Note the tall, smooth uniform appearance of these villi.



Photograph 28. Ileum-15, 25 days pi, infected piglet (L384-9), scanning EM
Note the presence of villous fusion and less organized appearance of these villi. 98.9X

compared to control values (Figures 13 and 14 and Photographs 24D and 23C). This was especially apparent within the jejunum-200 and lower-jejunum sections. No parasites in any pigs in any sections were seen.

Day 35 pi:

All piglets had an organized columnar intestinal epithelium. Villous length was not altered from that of the control pigs although the villi appeared wider (Figure 15). There was a very small amount of villous fusion present (Figure 20). There was a mild increase of intraepithelial lymphocytes within the villi along with a decrease of intraepithelial lymphocytes within the crypts. No other microscopic lesions were noted.

Microscopic lesions of the large intestine of infected pigs are described below:

At each age the large intestines were only slightly affected by the parasite infection. The parasite was seen both in the surface epithelium and in the crypt epithelium. There was very little, if any, inflammatory response associated with the presence of the parasites. Scattered focal microulcerations and occasional crypt abscesses were observed in the large intestinal sections, most frequently within the cecum. However, the same type of lesion was seen nearly as frequently in the control animals.

Morphometric Results

The results are summarized in Figures 15, 16, 17 and 18.

2 days pi:

There were no significant differences between the average villous length, the average crypt depth nor the villous/crypt ratio in any of the

measured locations.

5 days pi:

The villous lengths in all examined sections (duodenum, jejunum-200 and ileum-15) were all significantly ($p=0.05$) shorter than those of control pigs. The crypt depths were longer ($p=0.05$) in the jejunum and ileum. The villous/crypt ratio was significantly ($p=0.05$) decreased from those of control pigs in all sections.

9 days pi:

The villous length was significantly ($p=0.05$) shorter than those of control pigs in all sections examined. The crypt depths were longer ($P=0.05$) in the jejunum and ileum. The villous/crypt ratios were decreased in all sections.

15 days pi:

The villous lengths were significantly ($p=0.05$) shorter than controls in all sections yet they were significantly ($p=0.05$) longer than in infected pigs at 9 days pi. The crypt depths were longer ($p=0.05$) in the jejunum. The villous/crypt ratios were decreased ($p=0.05$) in the jejunum and ileum.

25 days pi:

The villous lengths were not different from those of control pigs. The crypt depths were generally shorter than those of control pigs, significantly ($p=0.05$) in the duodenal sections. The villous/crypt ratios were not different from those of control pigs.

35 days pi:

There were no significant differences between the villous lengths, crypt depths nor the villous/crypt ratios of infected piglets and those of control piglets.

Oocyst Counts

The oocyst counts of individual pigs measured at time of necropsy were maximal at 5 days pi with oocysts declining in numbers at 9, 15 and 25 days pi (Figure 38). The oocyst counts of individual pigs from two litters had a secondary peak at 15 days pi. The composite litter fecal oocyst counts are shown in Figure 29. Two peaks were found, the first and highest peak was found at 5-6 days pi. The second peak was found at 14-17 days pi.

Oocyst counts varied a great deal from pig to pig. At 5 days pi the variation ran from 688 oocysts/gram of washed fecal sediment to 3,960,351 oocysts/gram; at 9 days pi most feces contained less than 100 oocysts/gram but one pig shed 2,675 oocyst/gram. For individual pigs no correlation was evident between fecal oocyst counts and histologic lesions. However, the severity of histologic lesions found within a litter did correlate with the litter fecal oocyst counts.

Other Gastrointestinal Pathogens

No other gastrointestinal pathogens were found in any of the groups of piglets.

Figure 1. Villous Goblet Cells by Location at Days pi

The average number of goblet cells counted in ten villi per location per piglet identified by location at days pi in Isospora suis infected and control groups.

Location 1 = Duodenum, Location 2 = Jejunum-100, Location 3 = Jejunum-200,
Location 4 = Lower-Jejunum, Location 5 = Ileum-15, Location 6 = Ileum-5

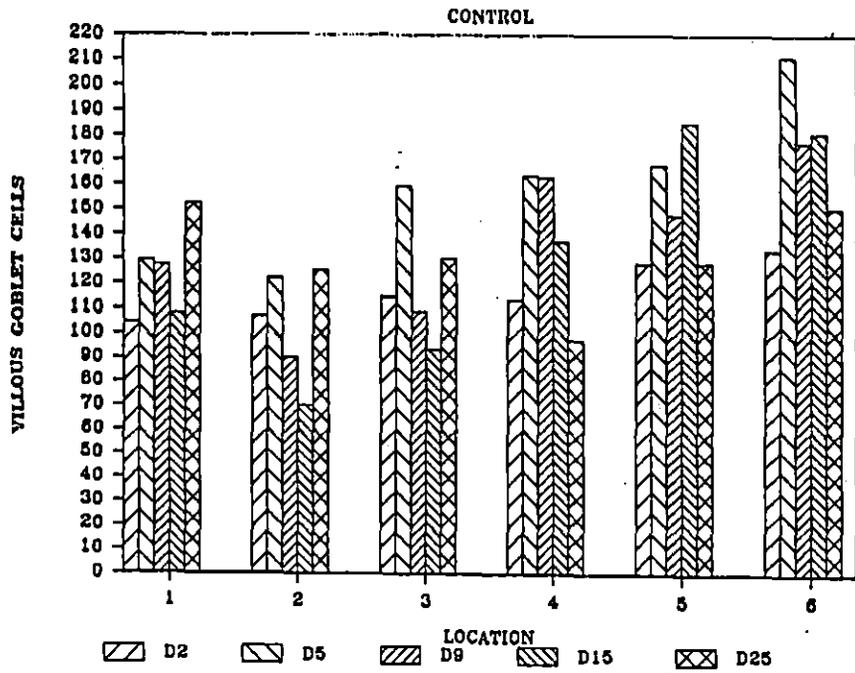
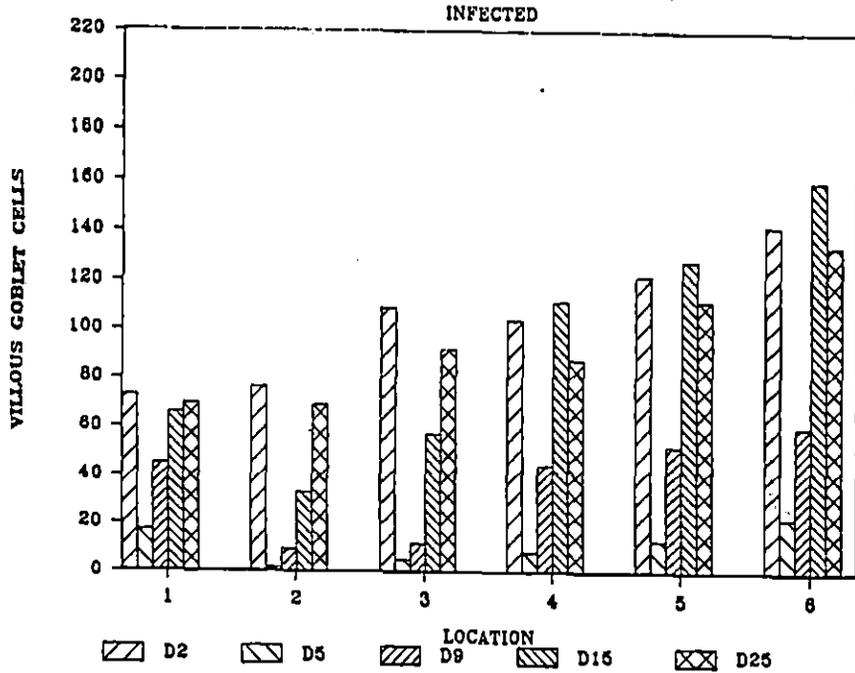


Figure 2. Crypt Goblet Cells by Location at Days pi

The average number of goblet cells counted in twenty crypts per location per piglet identified by location at day pi in Isospora suis infected and control groups.

Location 1 = Duodenum, Location 2 = Jejunum-100, Location 3 = Jejunum-200,
Location 4 = Lower-Jejunum, Location 5 = Ileum-15, Location 6 = Ileum-5,
Location 7 = Cecum, Location 8 = Proximal Colon, Location 9 = Spiral Colon

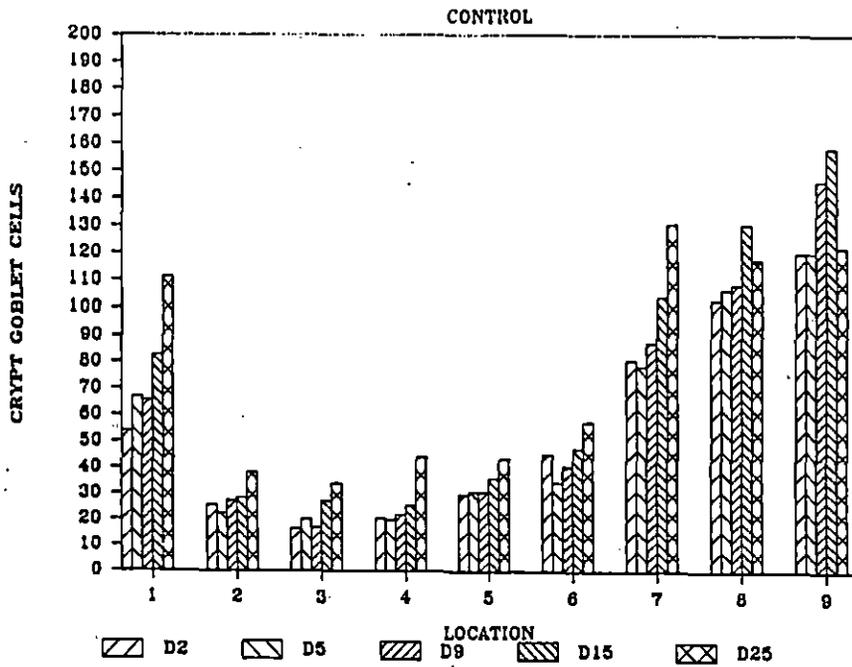
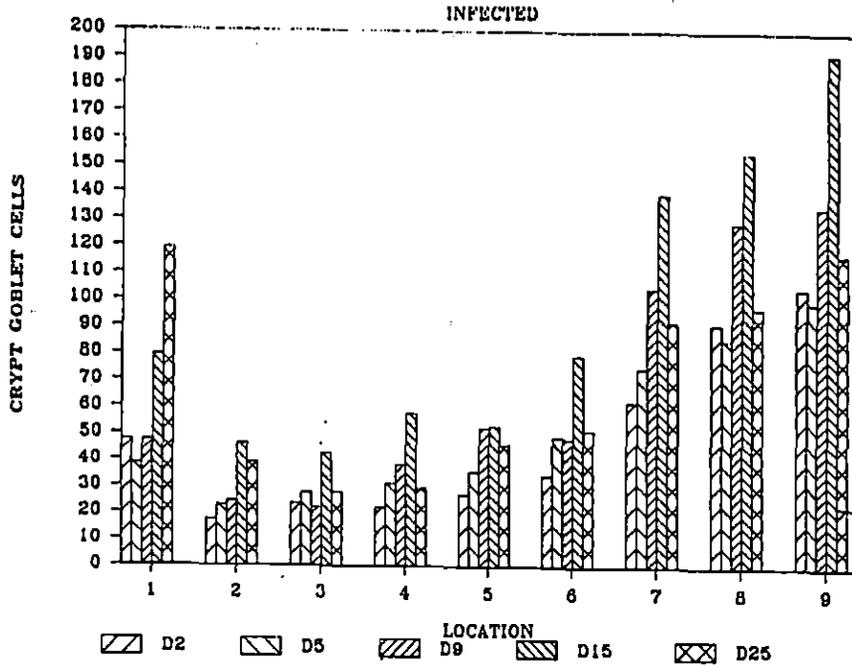


Figure 3. Villous Intraepithelial Lymphocytes by Location at Days pi

The average number of intraepithelial lymphocytes counted in ten villi per location per piglet identified by location at days pi in Isospora suis infected and control groups.

Location 1 = Duodenum, Location 2 = Jejunum-100, Location 3 = Jejunum-200,
Location 4 = Lower-Jejunum, Location 5 = Ileum-15, Location 6 = Ileum-5.

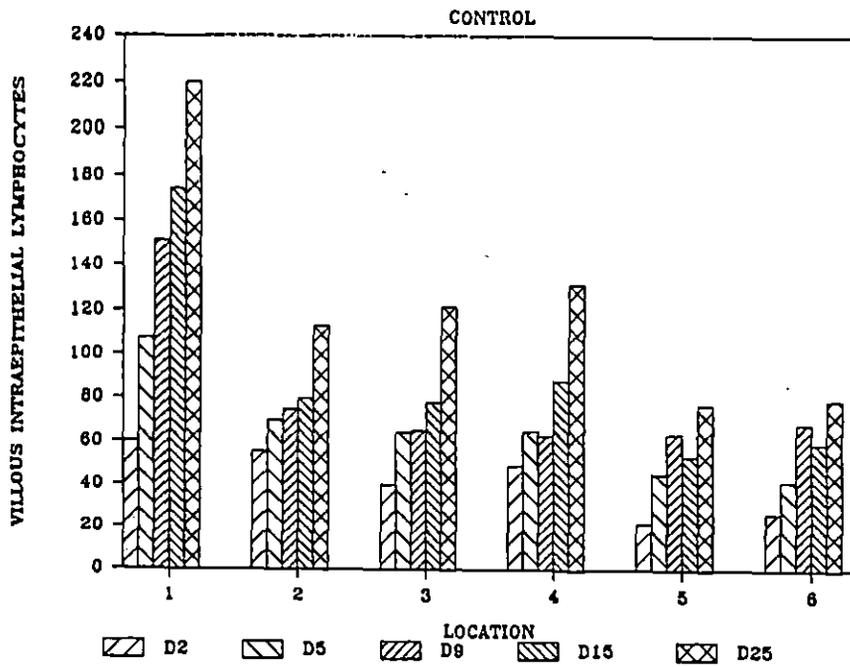
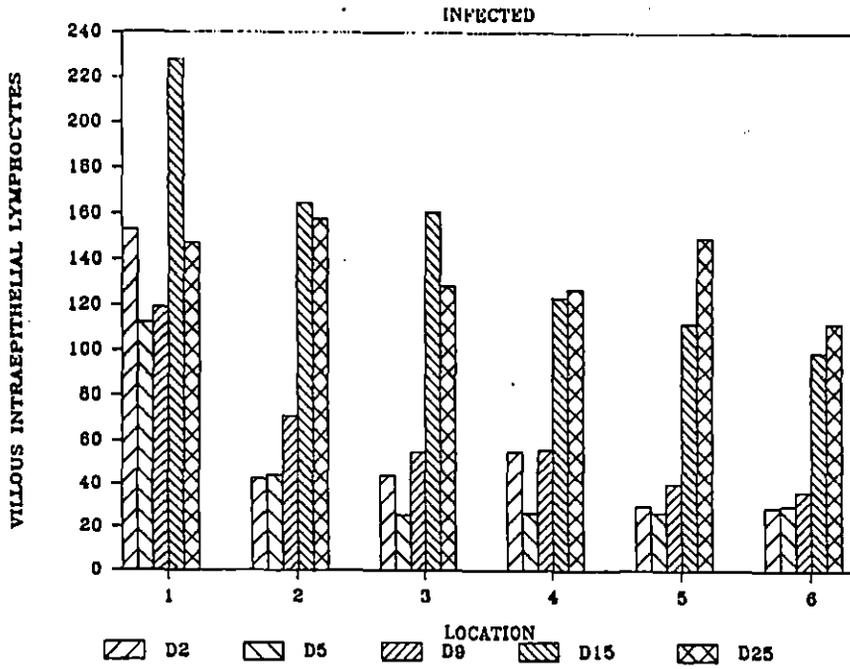
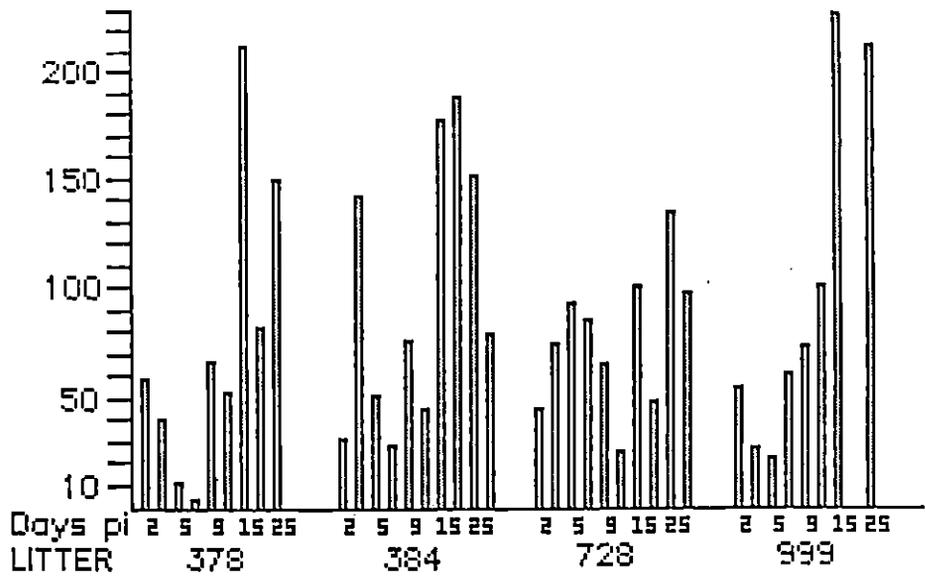


Figure 4. Villous Intraepithelial Lymphocytes by Infection Status and Days
pi

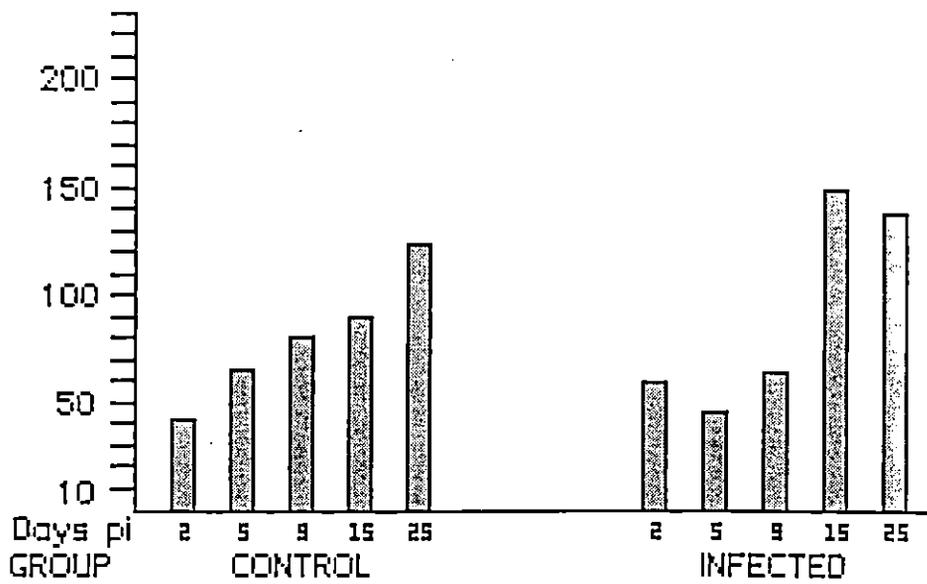
The average number of intraepithelial lymphocytes counted in ten villi per location per piglet through the course of infection in A.) individual piglets and B.) Isospora suis infected and control groups.

Number of INTRAEPITHELIAL LYMPHOCYTES in Villi
in Individual Infected Piglets



A

Number of INTRAEPITHELIAL LYMPHOCYTES in VILLI



B

Figure 5. Crypt Intraepithelial Lymphocytes by Location at Days pi

The average number of intraepithelial lymphocytes counted in twenty crypts per location per piglet identified by location at days pi in Isospora suis.

Location 1 = Duodenum, Location 2 = Jejunum-100, Location 3 = Jejunum-200,
Location 4 = Lower-Jejunum, Location 5 = Ileum-15, Location 6 = Ileum-5,
Location 7 = Cecum, Location 8 = Proximal Colon, Location 9 = Spiral Colon

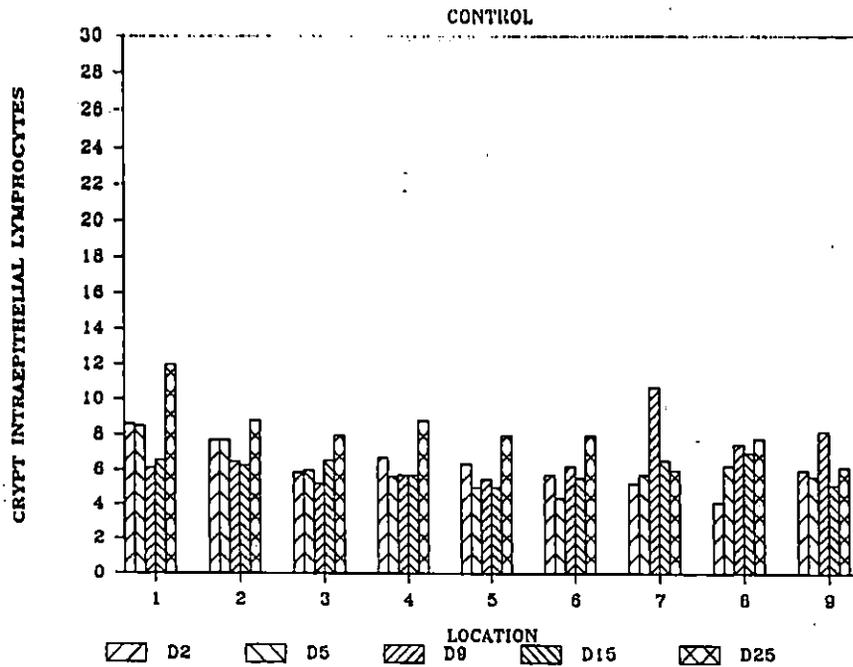
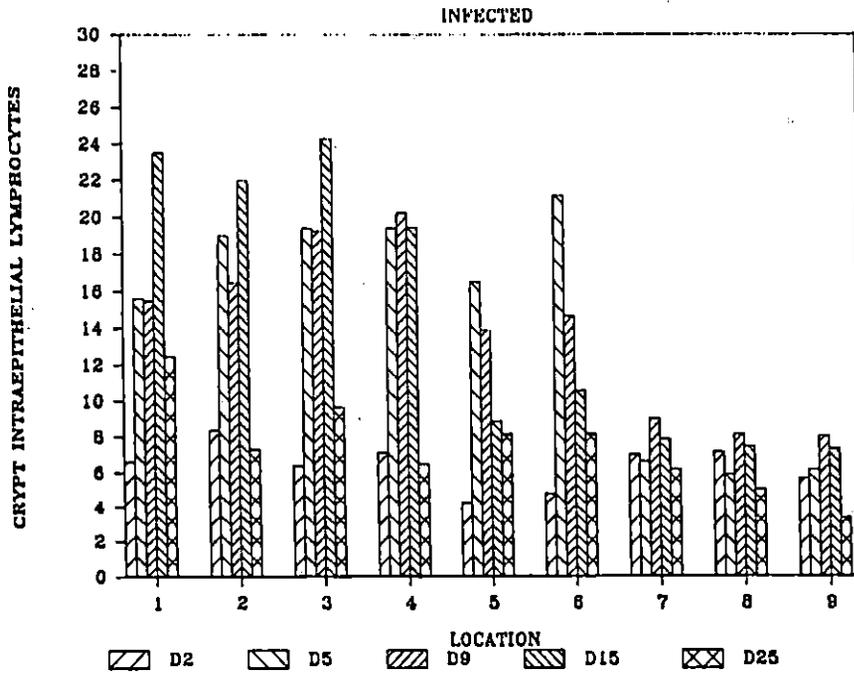


Figure 6. Crypt Intraepithelial Lymphocytes by Infection Status and by Days pi

The average number of intraepithelial lymphocytes counted in twenty crypts per location per piglet through the course of infection in A.) individual piglets and by days pi B.) Isospora suis infected and control groups.

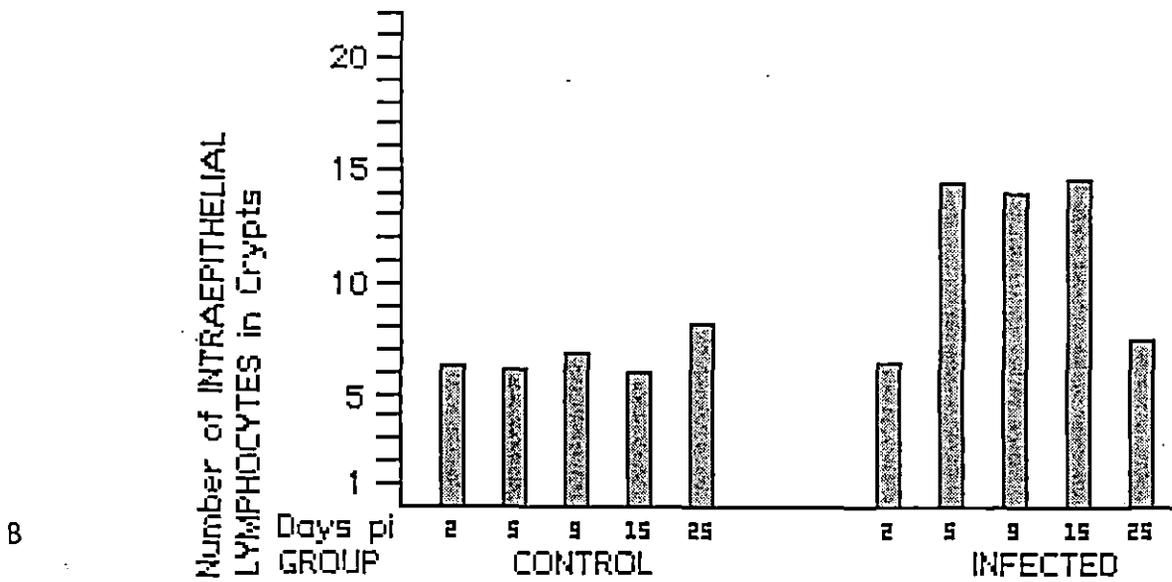
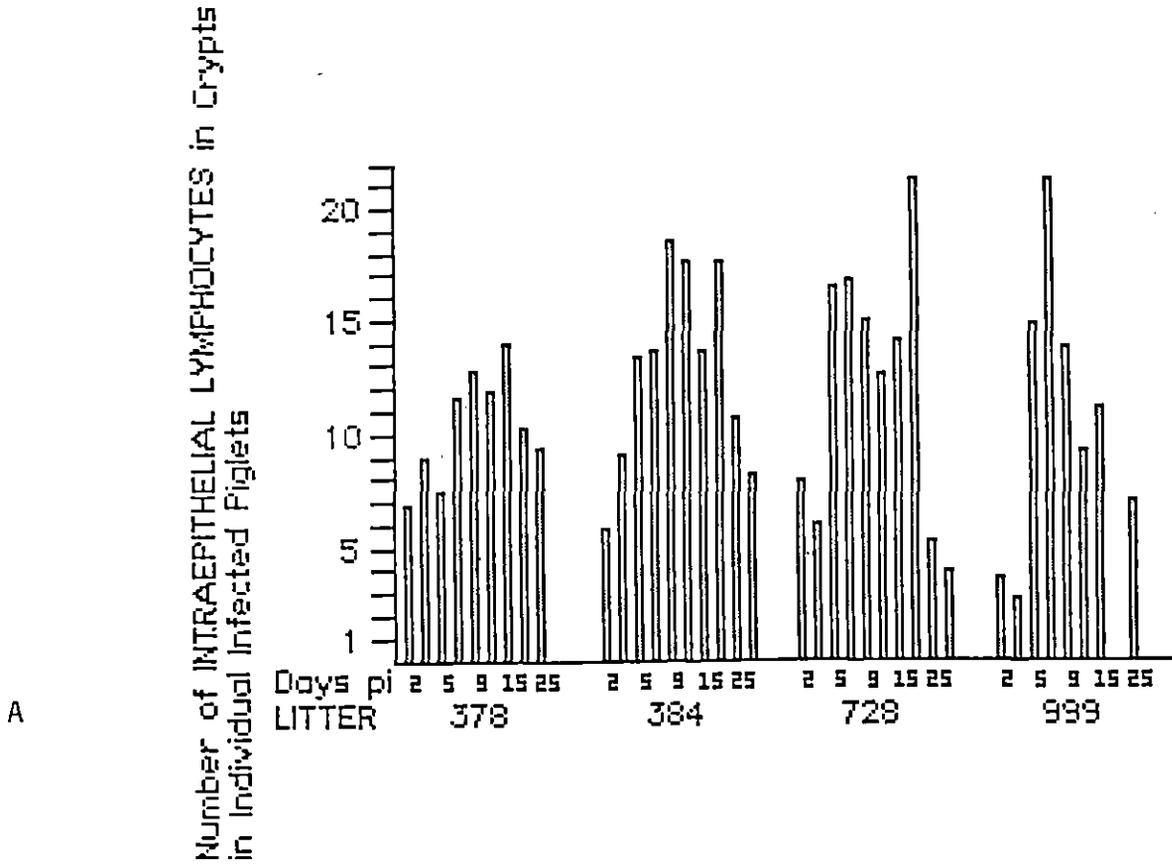


Figure 7. Villous Intraepithelial Neutrophils by Location at Days pi

The average number of intraepithelial neutrophils counted in ten villi per location per piglet identified by location at days pi in Isospora suis infected and control groups.

Location 1 = Duodenum, Location 2 = Jejunum-200, Location 3 = Jejunum-200,
Location 4 = Lower-Jejunum, Location 5 = Ileum-15, Location 6 = Ileum-5.

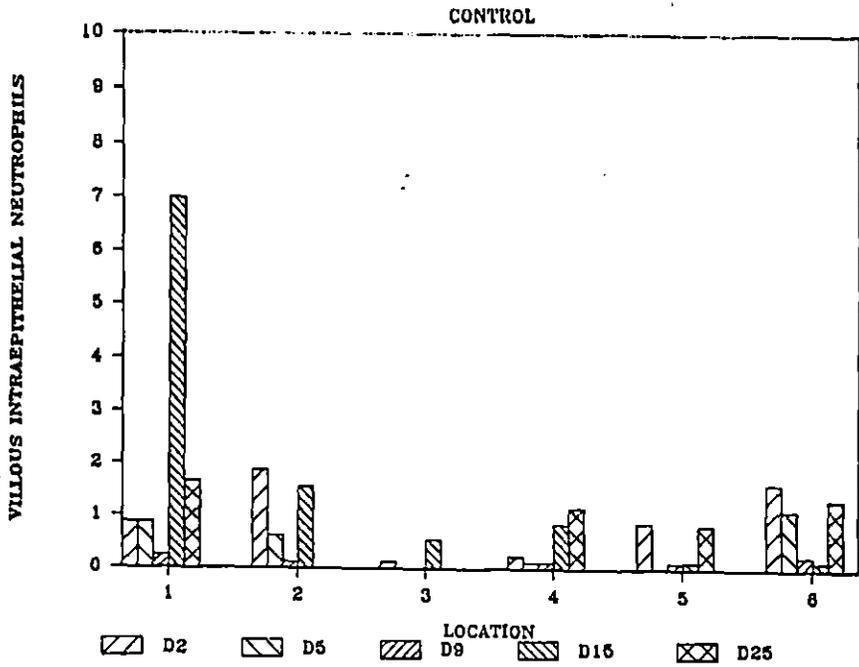
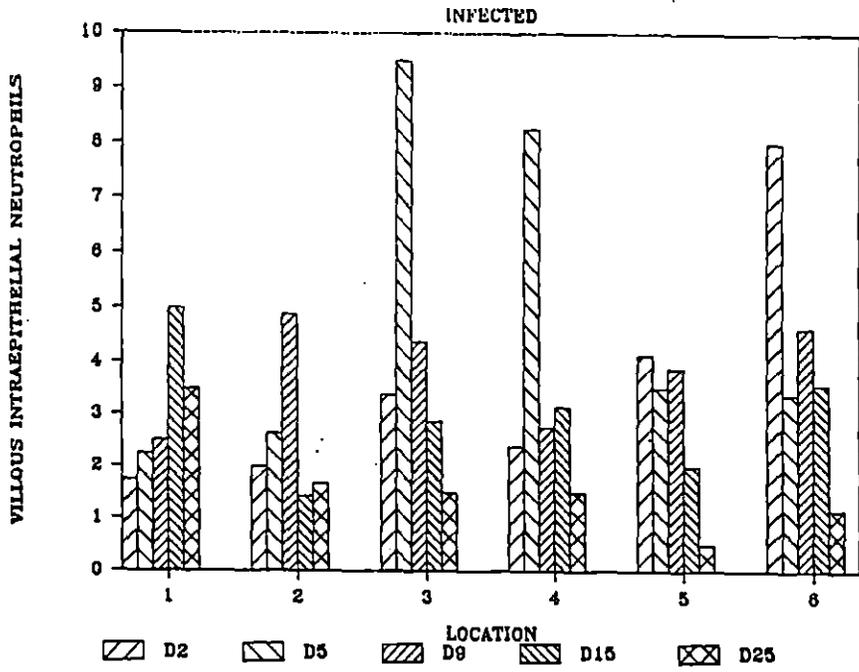


Figure 8. Villous Intraepithelial Neutrophils by Infection Status and by Day pi

The average number of intraepithelial neutrophils counted in ten villi per location per piglet through the course of infection in A.) individual infected piglets and B.) Isospora suis infected and control groups.

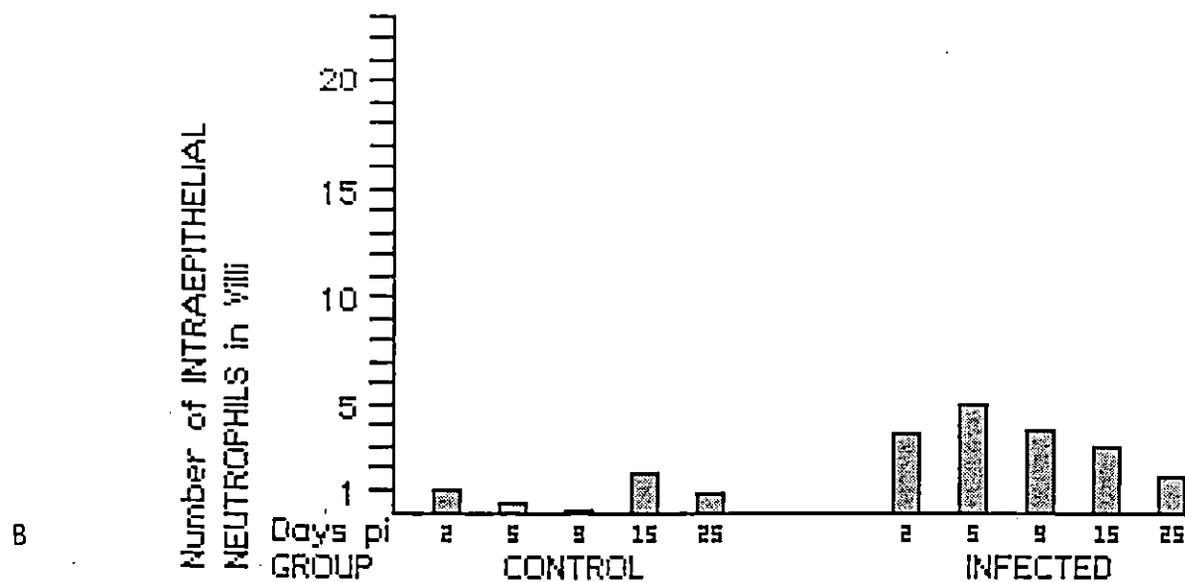
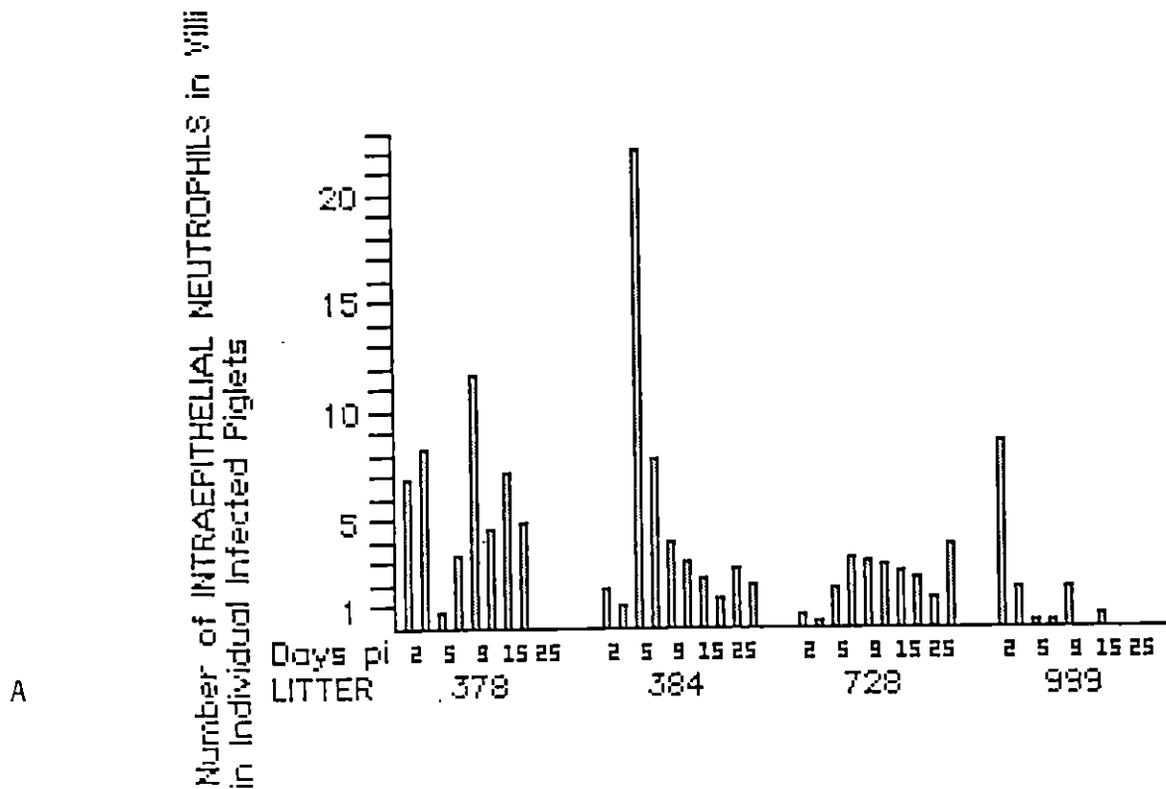


Figure 9. Lamina Propria Neutrophils by Location at Days pi

The average number of neutrophils counted within the lamina propria of five high-powered fields per location per piglet identified by location at days pi in Isospora suis infected and control groups.

Location 1 = Duodenum, Location 2 = Jejunum-100, Location 3 = Jejunum-200,
Location 4 = Lower-Jejunum, Location 5 = Ileum-15, Location 6 = Ileum-5.

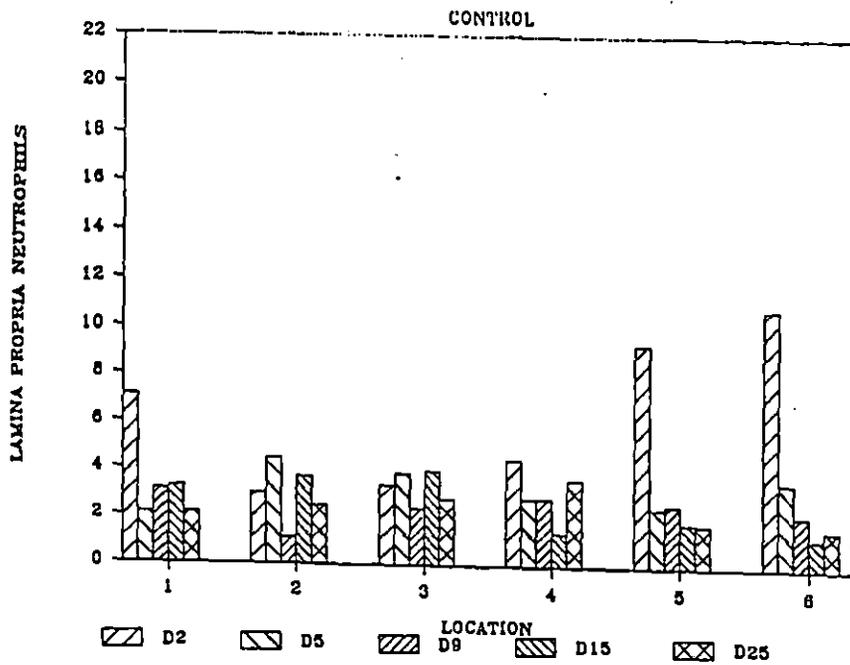
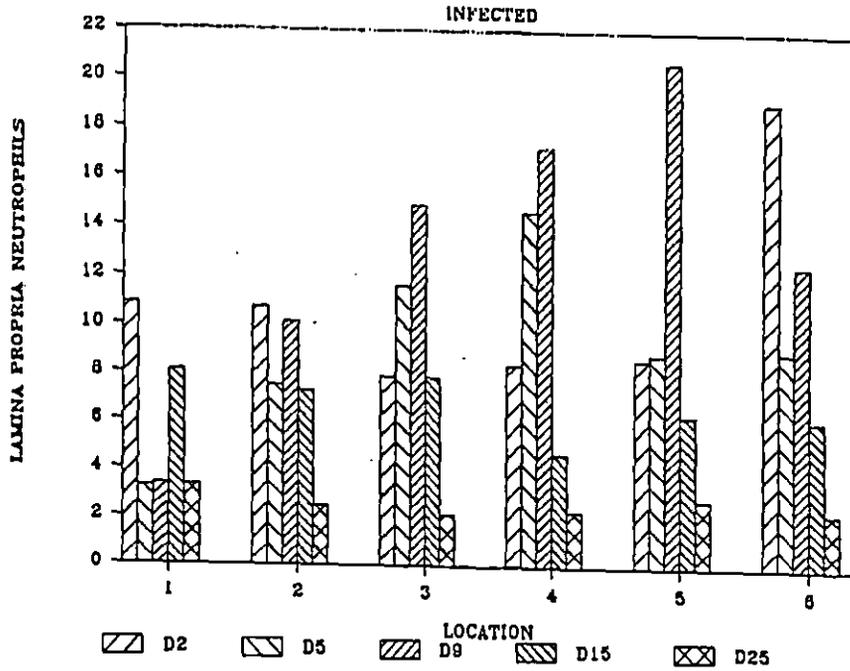
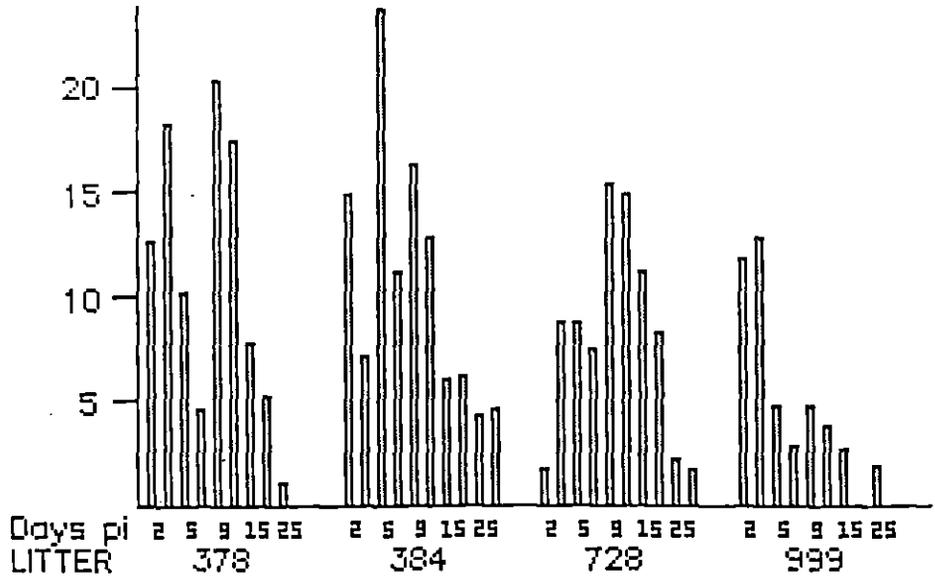


Figure 10. Lamina Propria Neutrophils by Infection Status and by Days pi

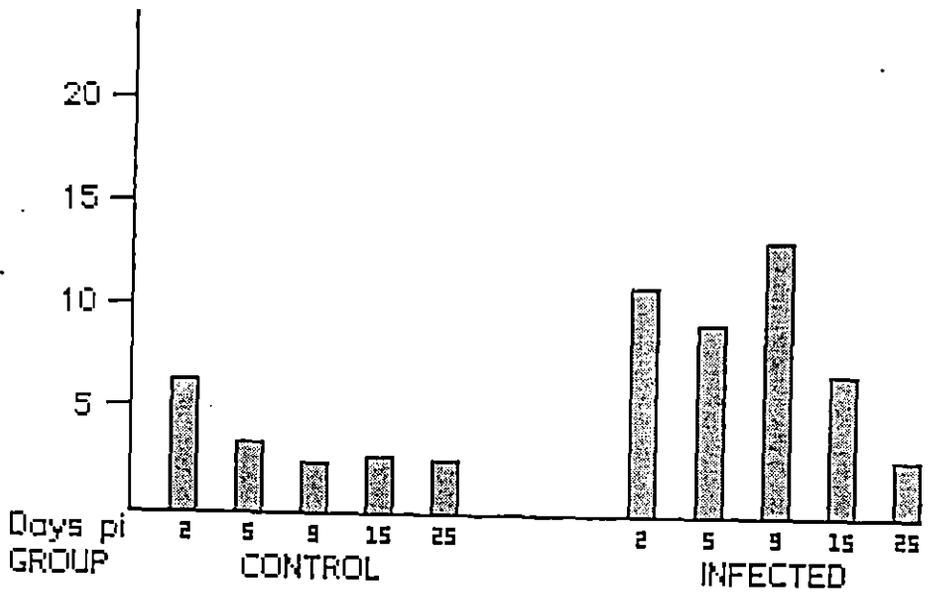
The average number of neutrophils counted within the lamina propria of five high-powered fields per location per piglet through the course of infection in A.) individual infected piglets and B.) Isospora suis infected and control groups.

Number of NEUTROPHILS in the Lamina Propria in Individual Infected Piglets



A

Number of NEUTROPHILS in the Lamina Propria



B

Figure 11. Lamina Propria Eosinophils by Location at Days pi

The average number of eosinophils within the lamina propria of five high-powered fields per location per piglet identified by location at days pi in Isospora suis infected and control groups.

Location 1 = Duodenum, Location 2 = Jejunum-100, Location 3 = Jejunum-200, Location 4 = Lower-Jejunum, Location 5 = Ileum-15, Location 6 = Ileum-5.

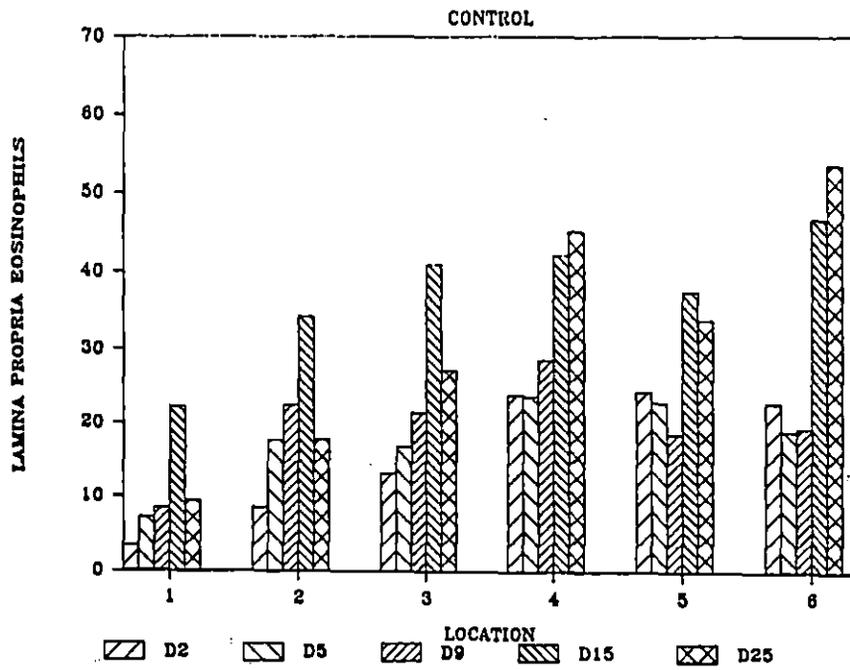
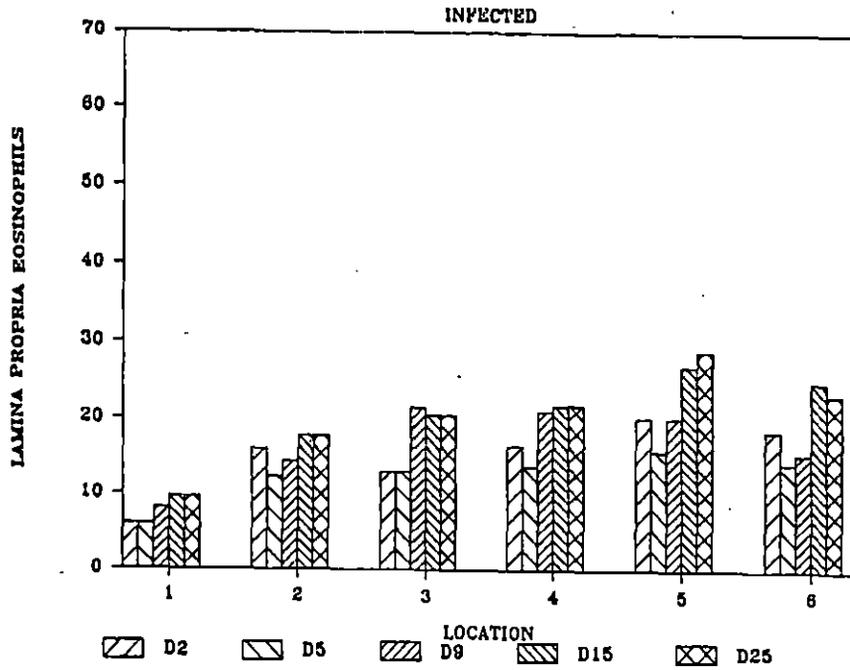
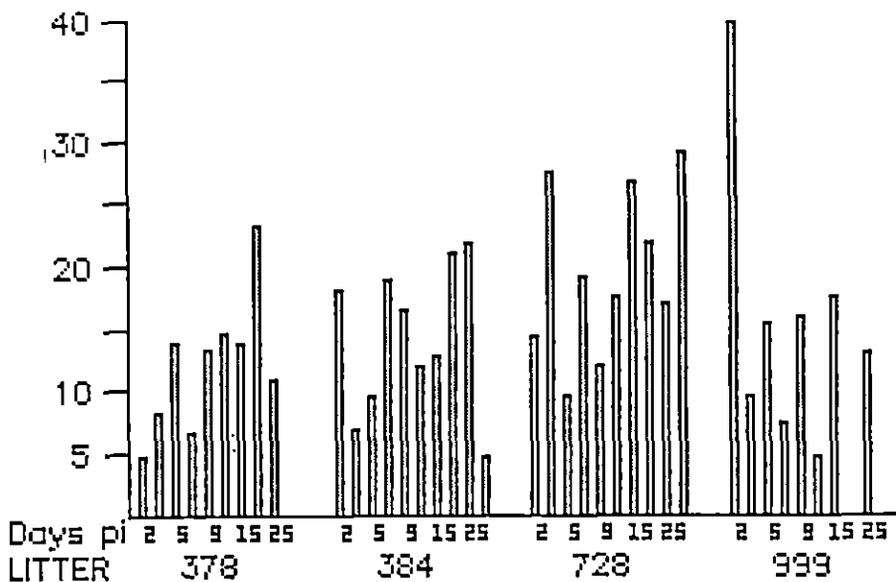


Figure 12. Lamina Propria Eosinophils by Infection Status and by Days pi

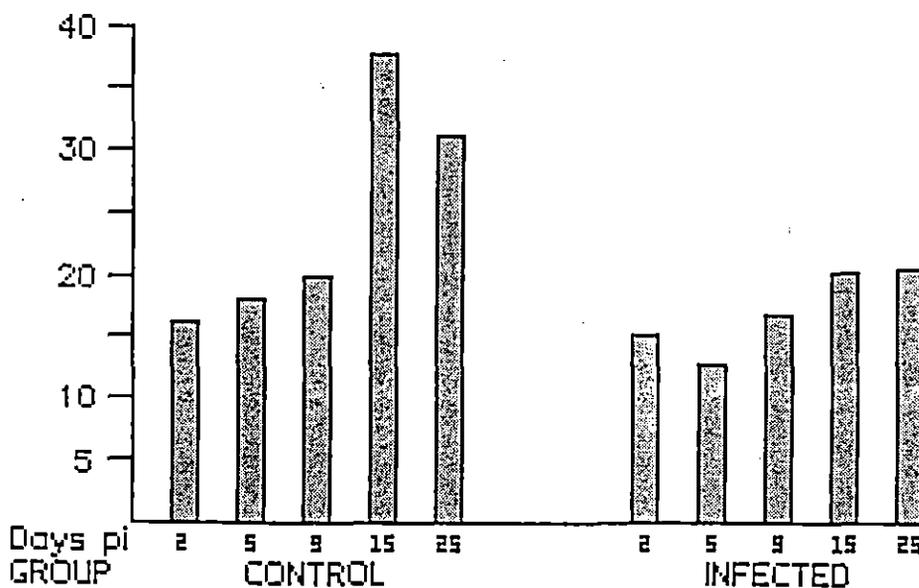
The average number of eosinophils counted within the lamina propria of five high-powered fields per location per piglet through the course of infection in A.) individual infected piglets and B.) Isospora suis infected and control groups.

Number of EOSINOPHILS in the Lamina Propria in Individual Infected Piglets



A

Number of EOSINOPHILS in Lamina Propria



B

Figure 13. Mitotic Index by Location at Days pi

The average number of mitotic figures counted in twenty crypts per location per piglet identified by location at days pi in Isospora suis infected and control groups.

Location 1 = Duodenum, Location 2 = Jejunum-100, Location 3 = Jejunum-200,
Location 4 = Lower-Jejunum, Location 5 = Ileum-15, Location 6 = Ileum-5,
Location 7 = Cecum, Location 8 = Proximal Colon, Location 9 = Spiral Colon.

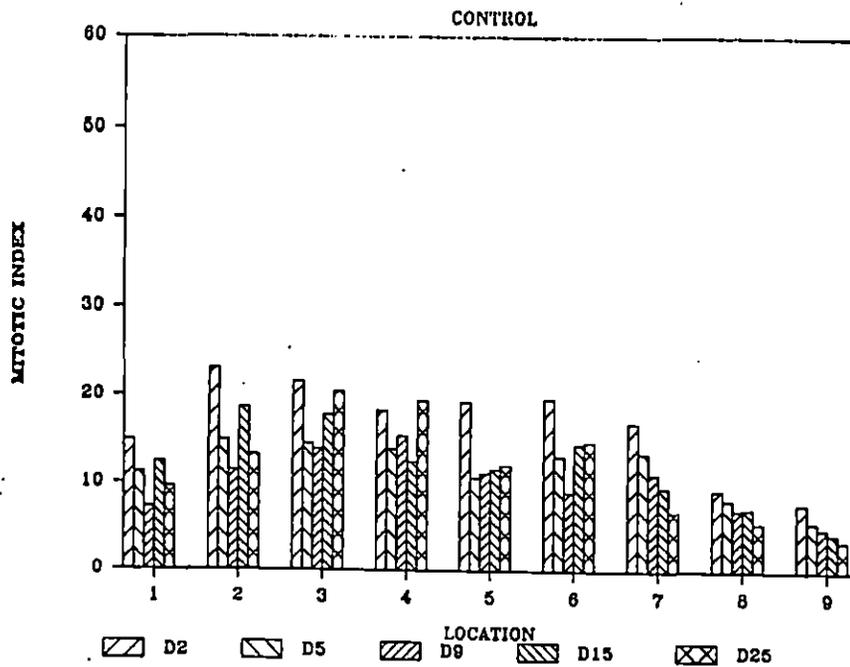
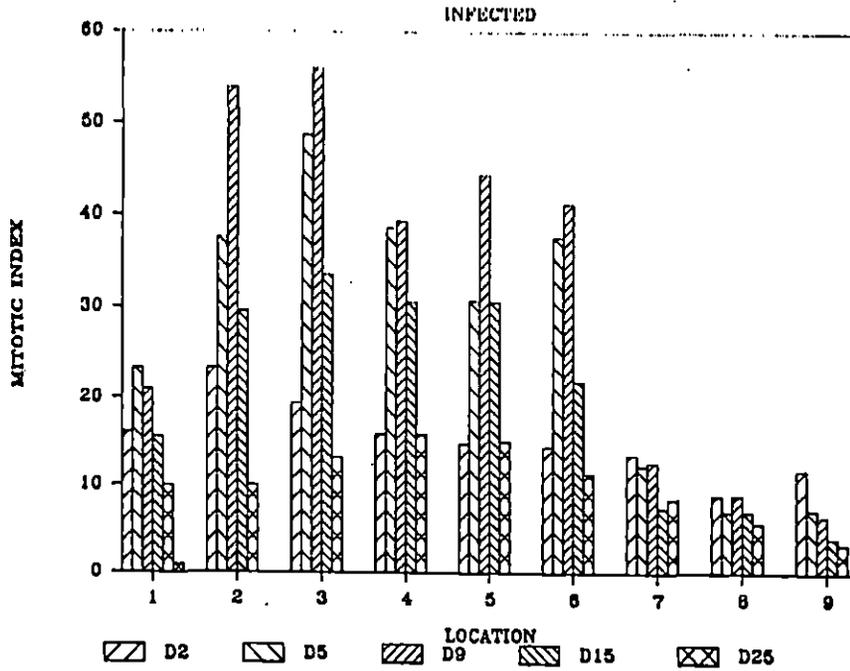
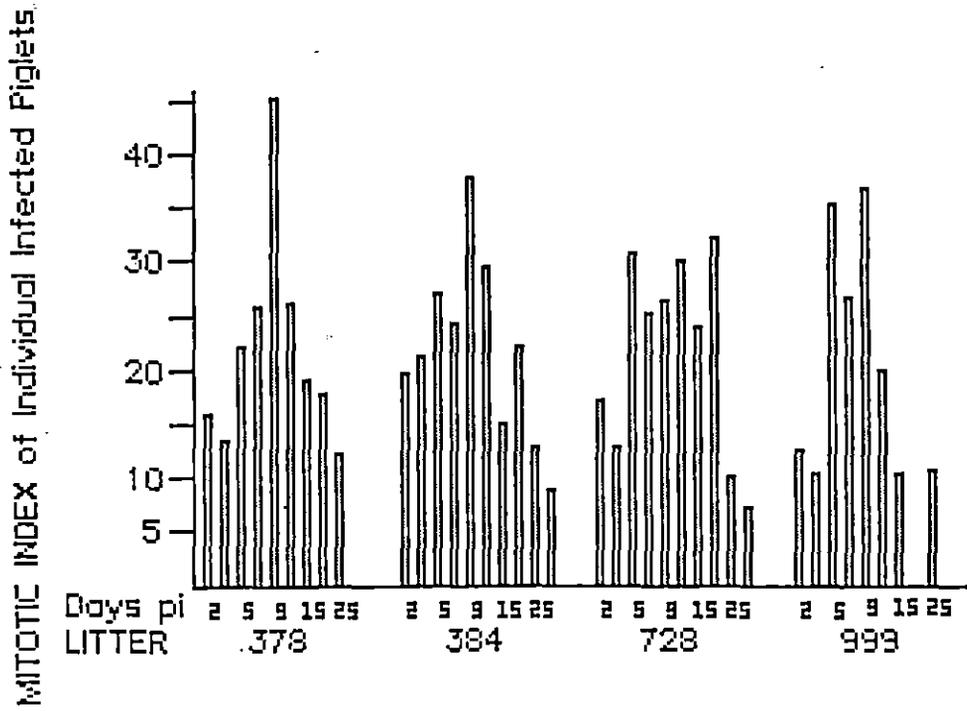


Figure 14. Mitotic Index by Infection Status and by Days pi

The average number of mitotic figures counted in twenty crypts per location per piglet through the course of infection in A.) individual infected piglets and B.) Isospora suis infected and control groups.

A



B

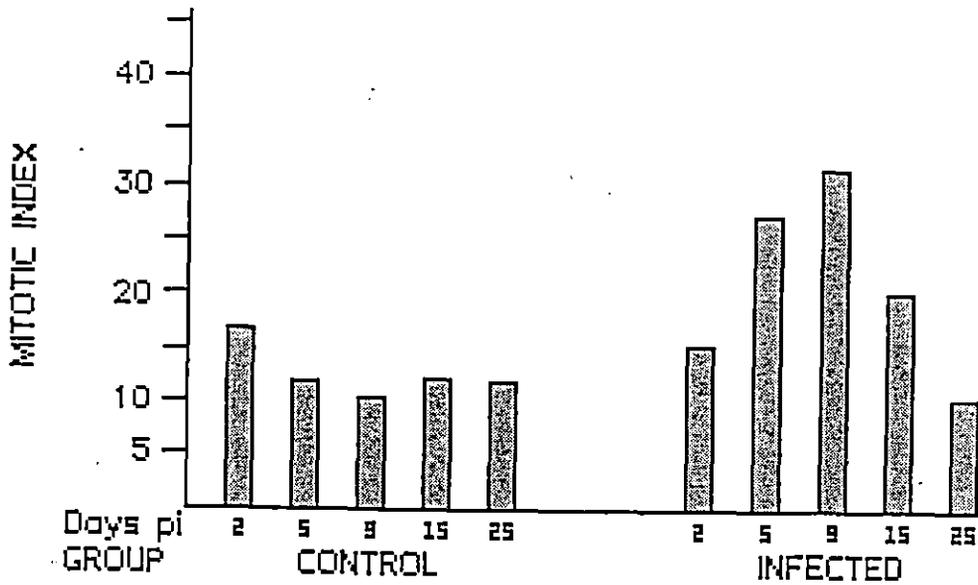


Figure 15. Villous Length by Location at Days pi

The average villous length (in microns) measured by morphometric techniques identified by location at days pi in Isospora suis infected and control groups.

Location 1 = Duodenum, Location 2 = Jejunum-200, Location 3 = Ileum-15.

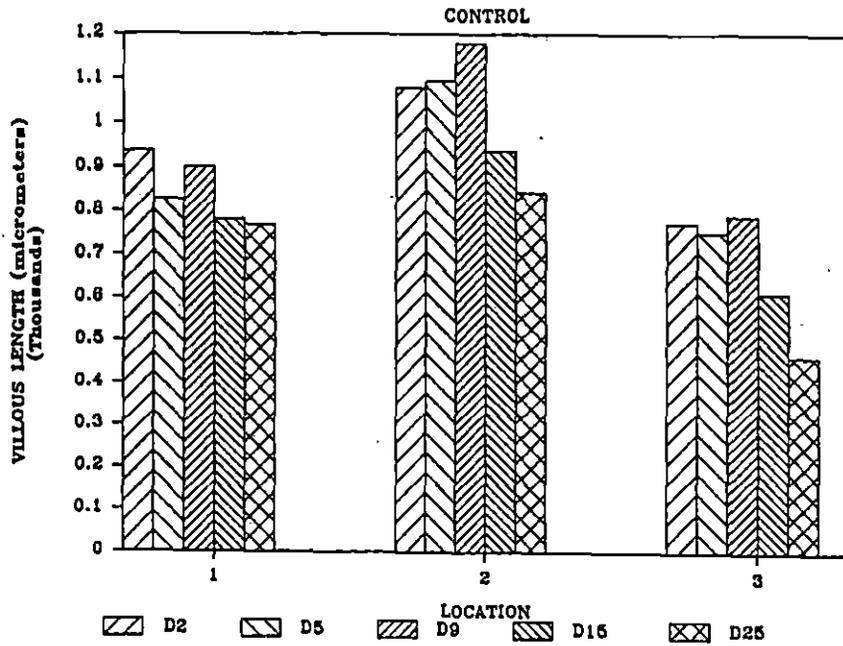
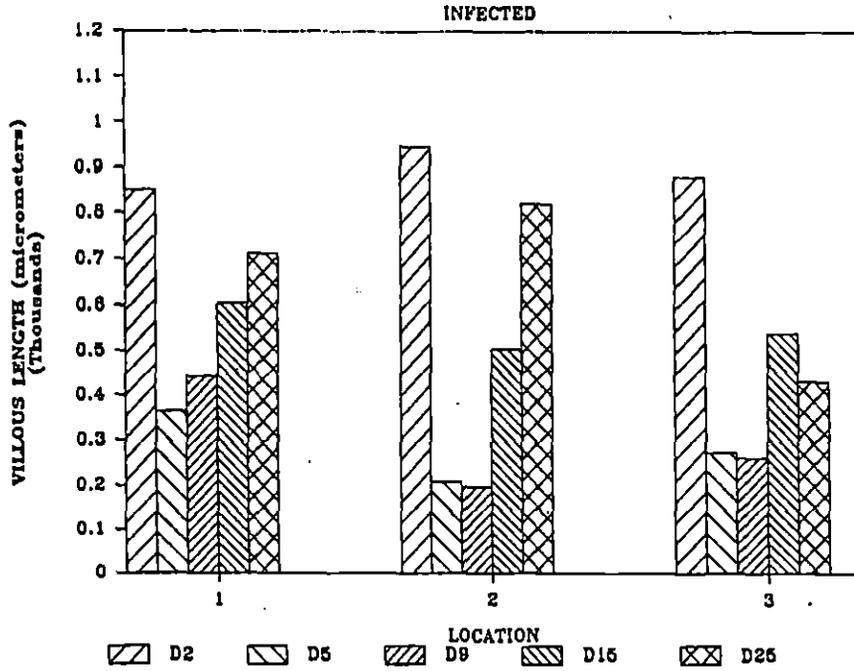
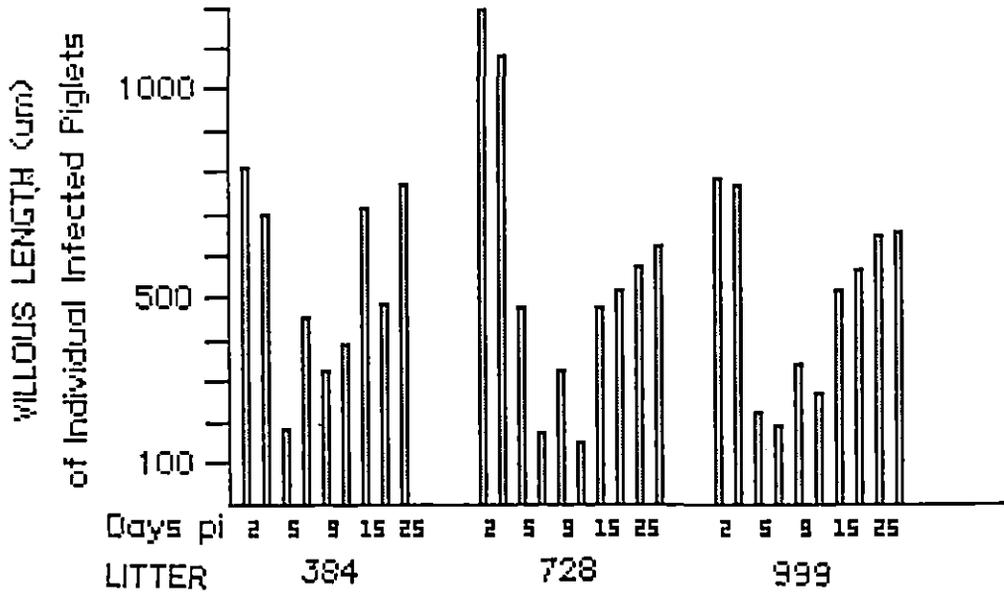
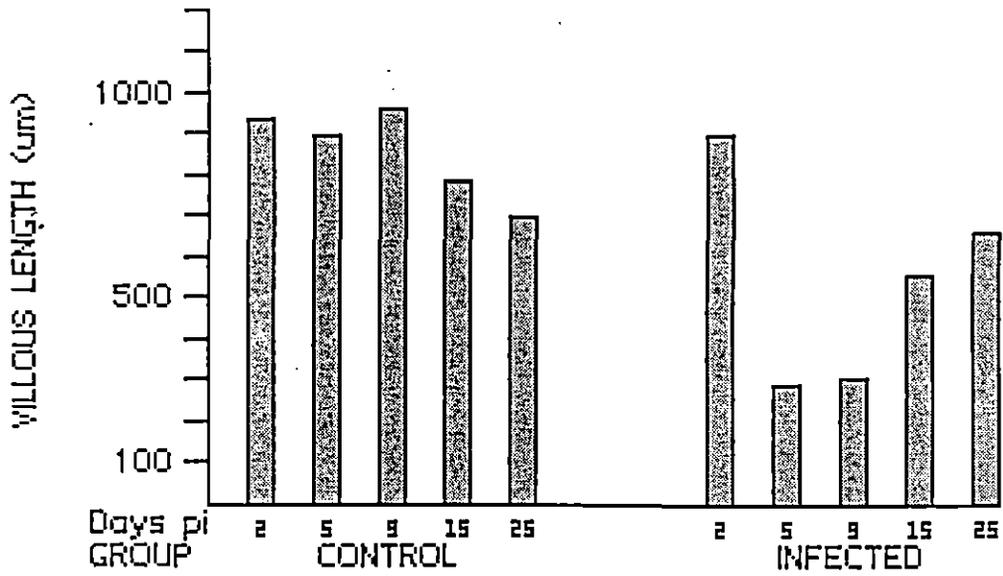


Figure 16. Villous Length by Infection Status and by Days pi

The average villous length (in microns) measured by morphometric techniques through the course of infection in A.) individual infected piglets and B.) Isospora suis infected and control groups.



A



B

Figure 17. Crypt Depth by Location at Days pi

The average crypt depth (in microns) measured by morphometric techniques identified by location at days pi in Isospora suis infected and control groups.

Location 1 = Duodenum, Location 2 = Jejunum-200, Location 3 = Ileum-15.

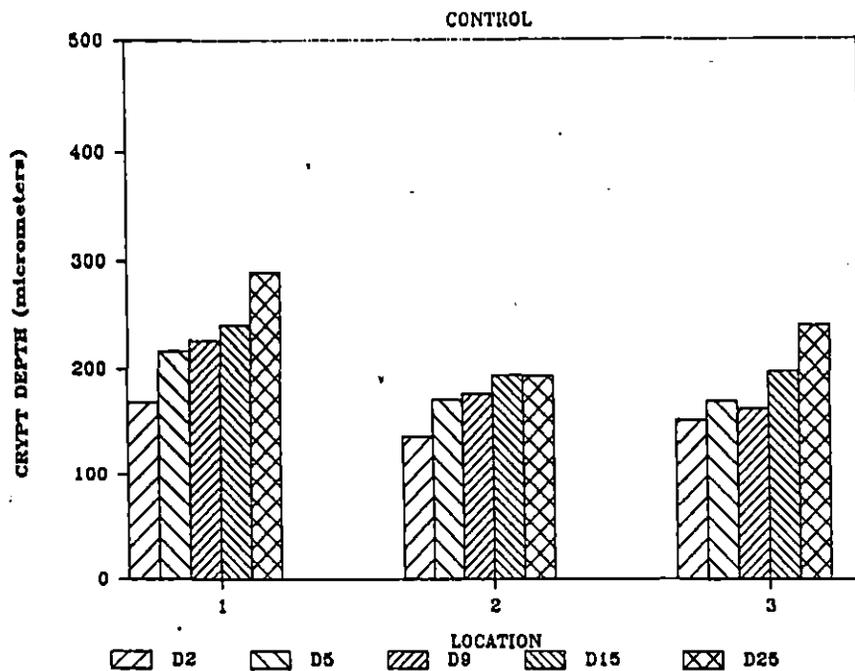
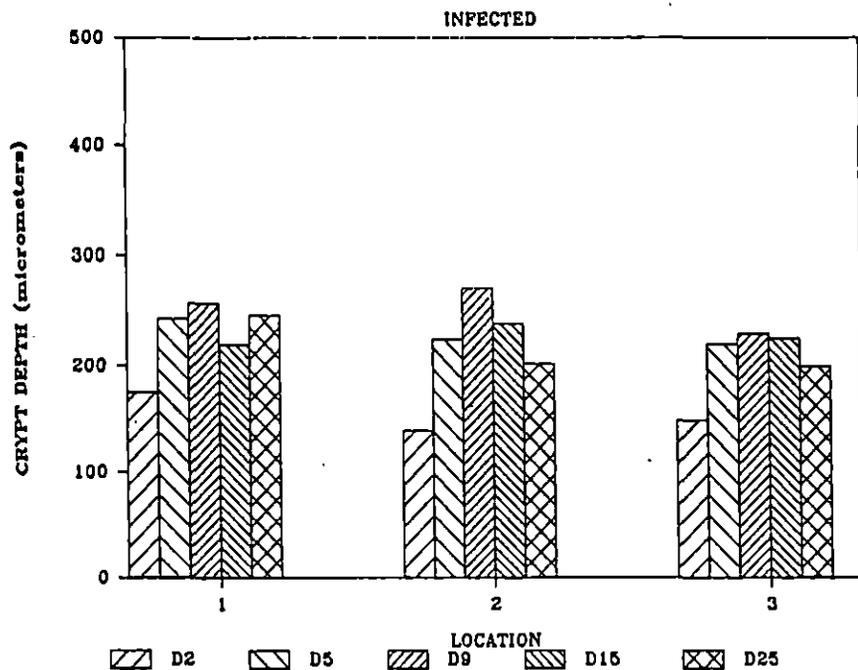


Figure 18. Villous Length/Crypt Depth Ratio

The average villous length/crypt depth ratio measured by morphometric techniques identified by location at days pi in Isospora suis infected and control groups.

Location 1 = Duodenum, Location 2 = Jejunum-200, Location 3 = Ileum-15.

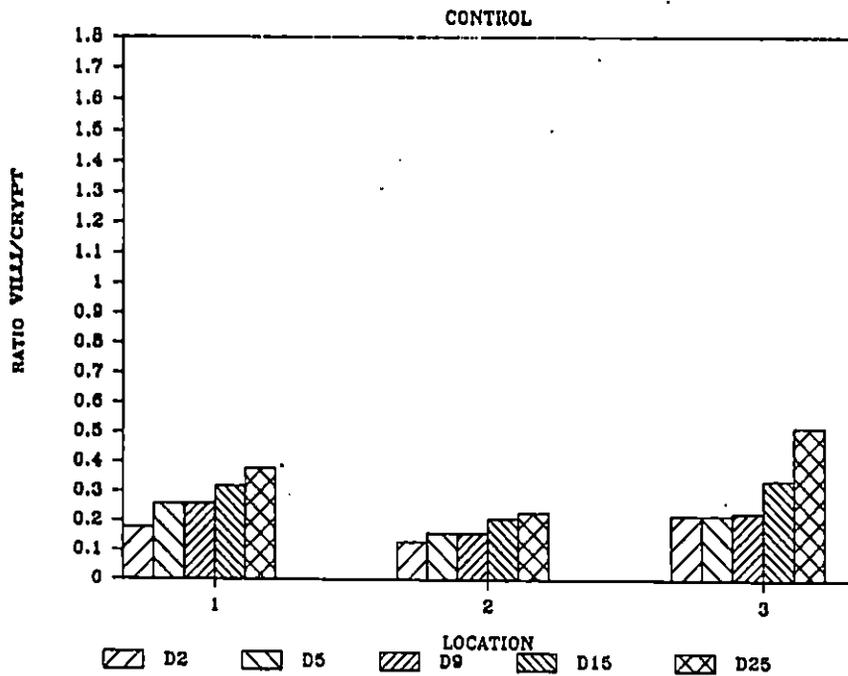
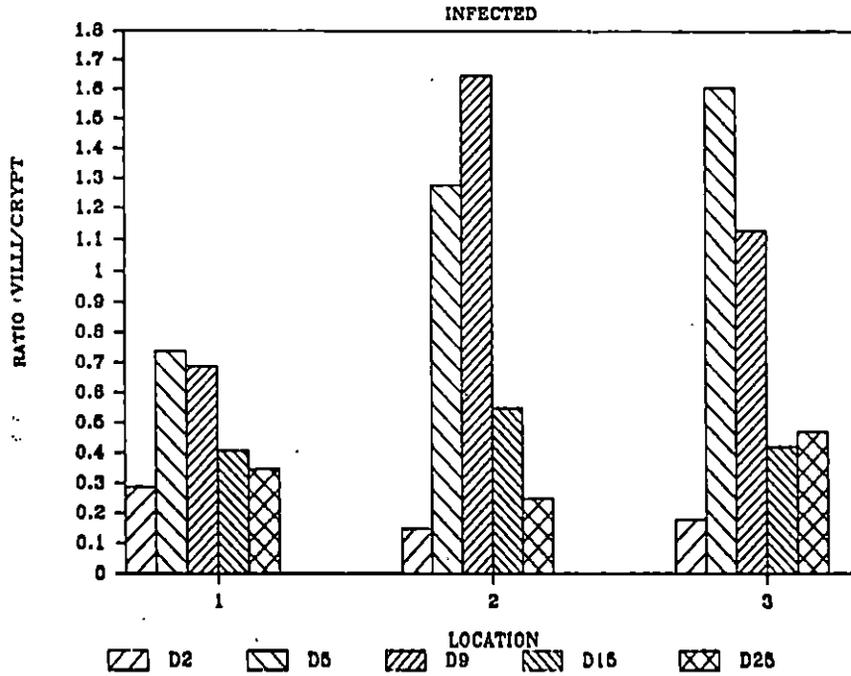


Figure 19: Villous Epithelial Focal Necrosis

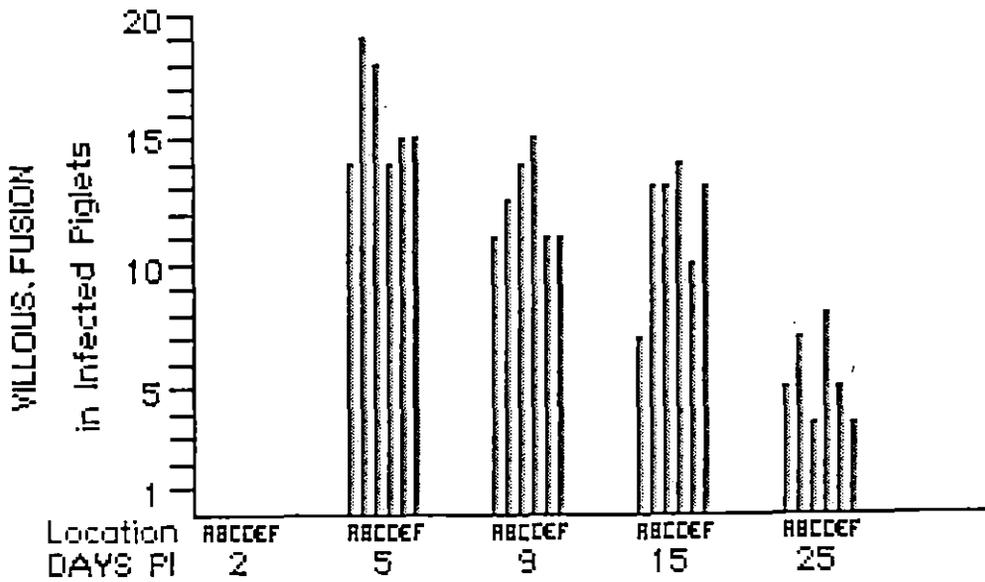
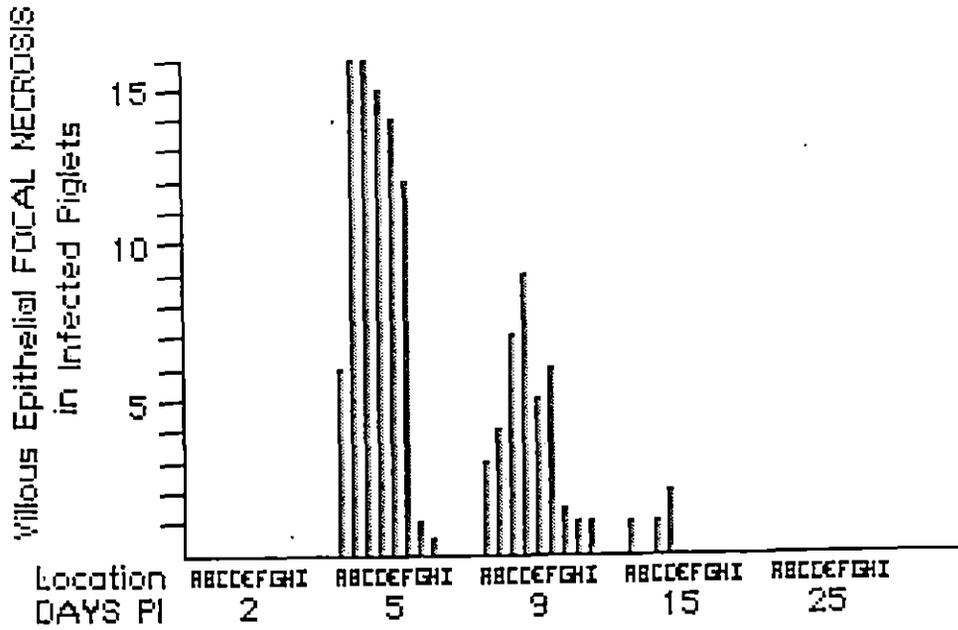
The average number of villous epithelial focal necrosis observed in Isospora suis infected piglets identified by location of intestine at days pi.

Location A = Duodenum, Location B = Jejunum-100, Location C = Jejunum-200,
Location D = Lower-Jejunum, Location E = Ileum-15, Location F = Ileum-5,
Location G = Cecum, Location H = Proximal Colon, Location I = Spiral Colon

Figure 20. Villous Fusion

The average amount of villous fusion observed in Isospora suis infected piglets identified by location of intestine at days pi.

Location A = Duodenum, Location B = Jejunum-100, Location C = Jejunum-200,
Location D = Lower-Jejunum, Location E = Ileum-15, Location F = Ileum-5



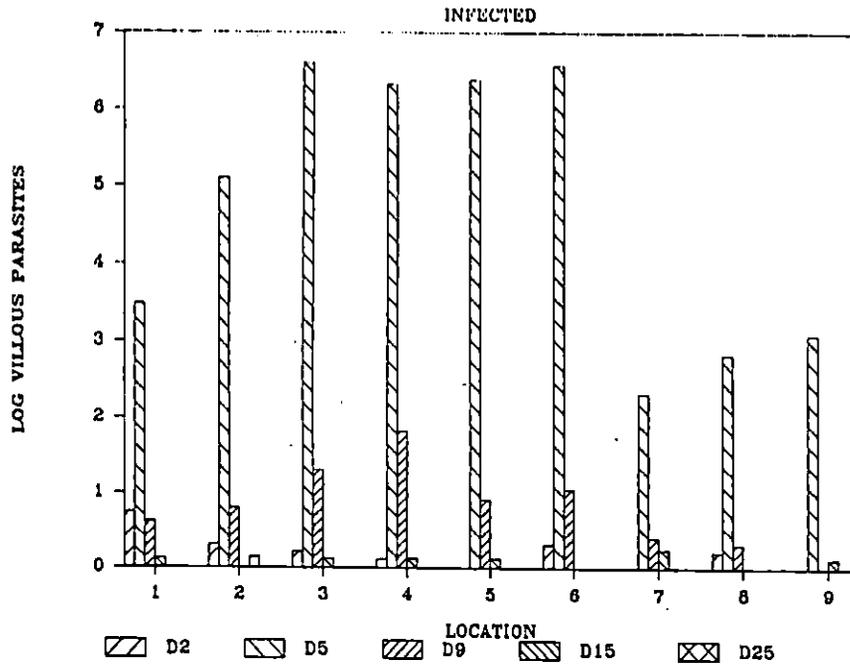


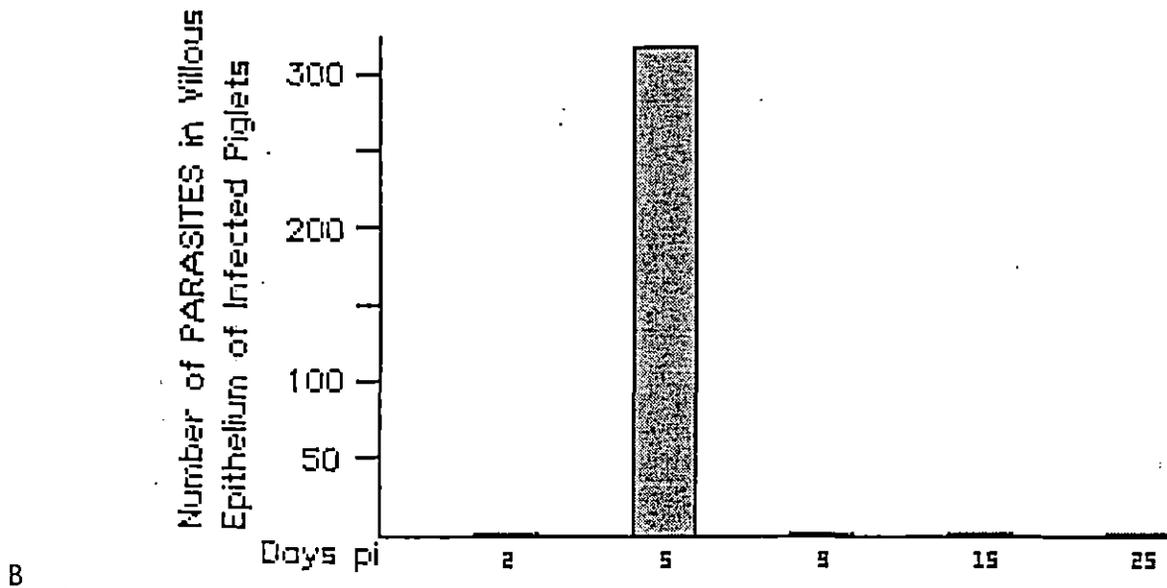
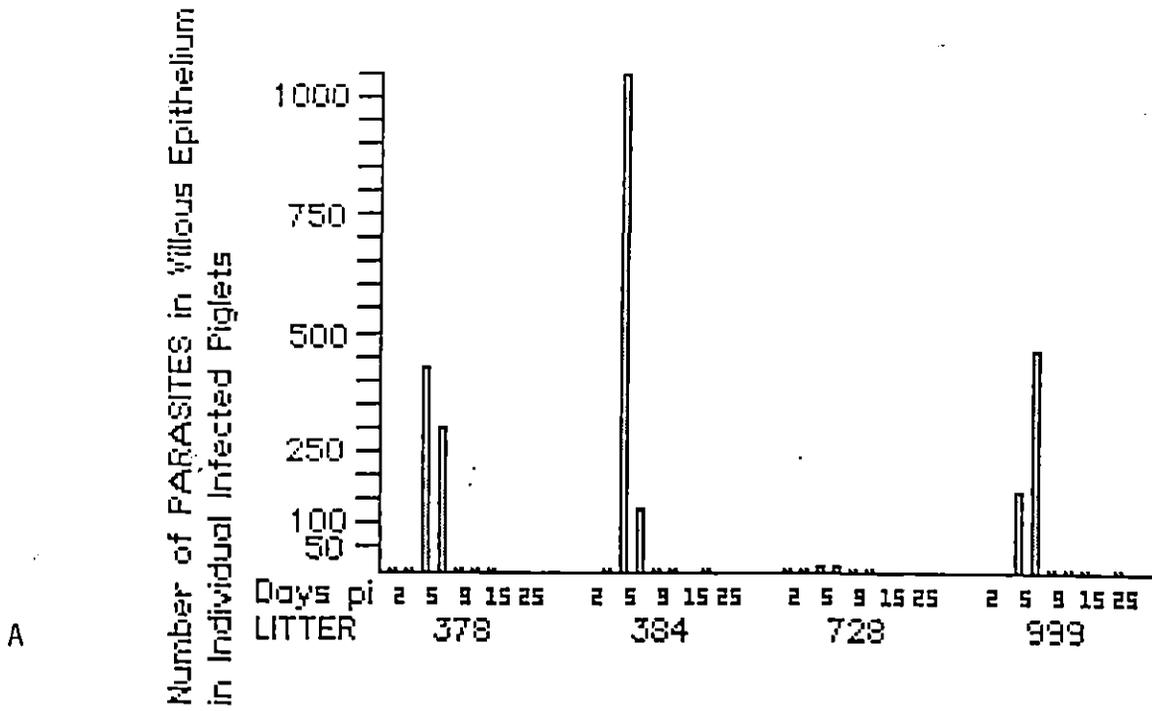
Figure 21. Log of Villous Parasites by Location at Days pi

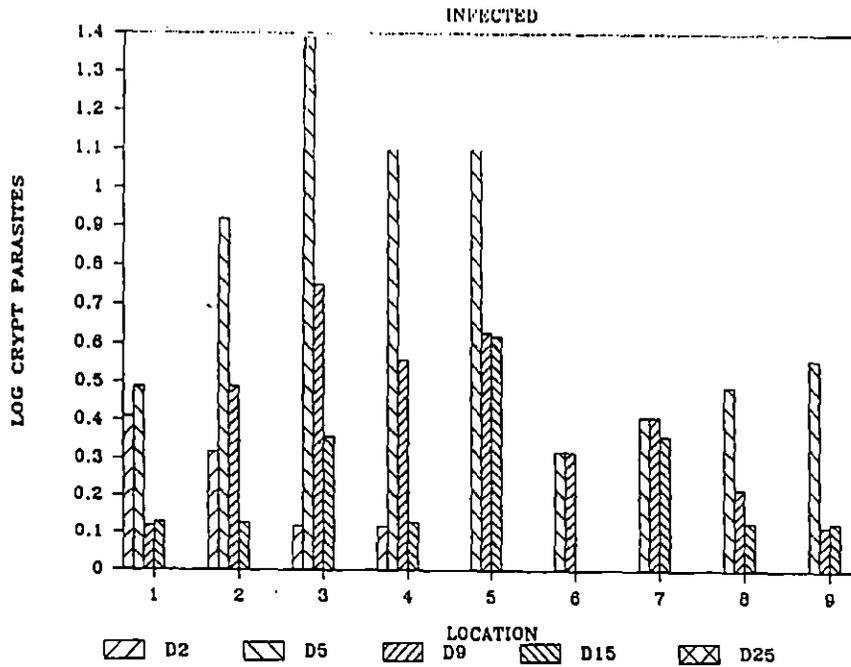
The log of the average number of parasites counted in the loop section of every location in each infected piglet identified by location at days pi.

Location 1 = Duodenum, Location 2 = Jejunum-100, Location 3 = Jejunum-200, Location 4 = Lower-Jejunum, Location 5 = Ileum-15, Location 6 = Ileum-5.

Figure 22. Villous Parasites by Infection Status and by Days pi

The average number of parasites counted in the loop section of every location in each infected piglet through the course of infection in A.) individual infected piglets and B.) Isospora suis infected group.





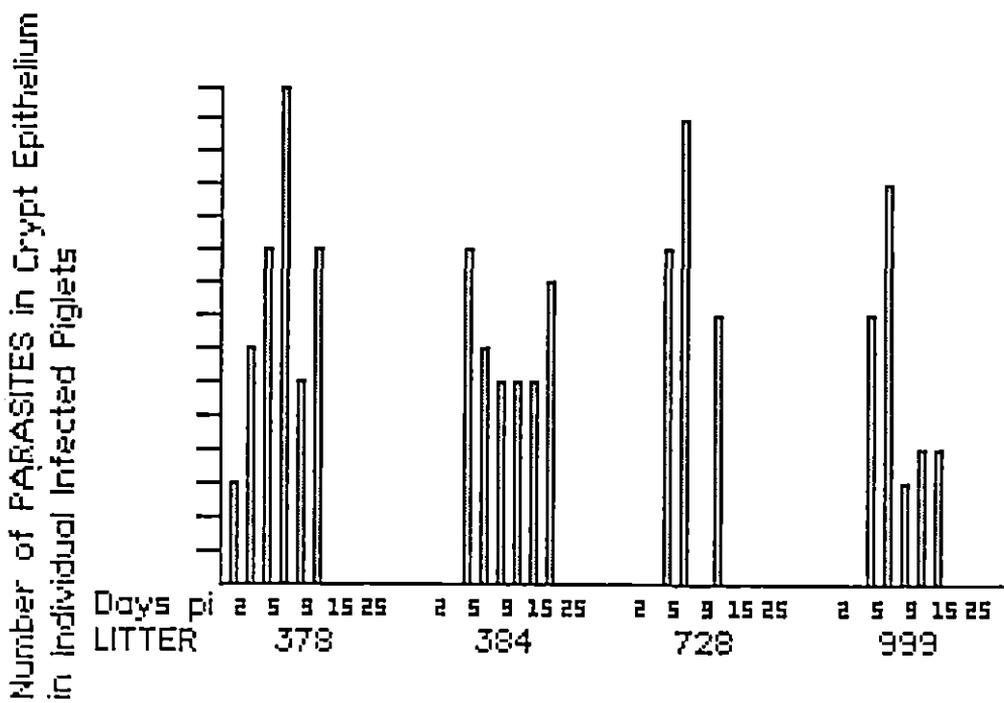
Location 1 = Duodenum, Location 2 = Jejunum-100, Location 3 = Jejunum-200, Location 4 = Lower-Jejunum, Location 5 = Ileum-15, Location 6 = Ileum-5, Location 7 = Cecum, Location 8 = Proximal Colon, Location 9 = Spiral Colon.

Figure 23. Log of Crypt Parasites by Location at Days pi

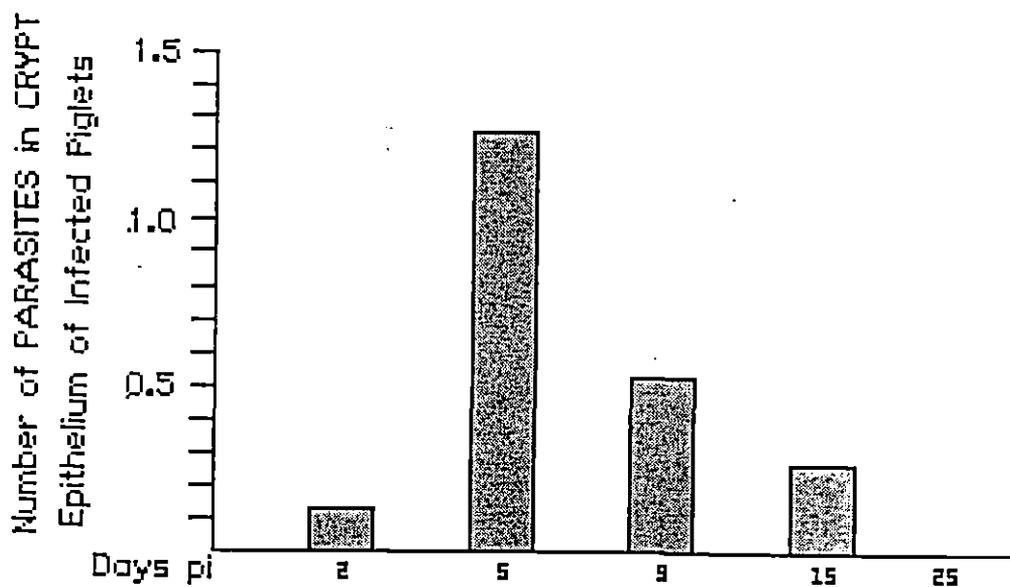
The average number of parasites observed in the loop section of every location in each infected piglet identified by location at days pi.

Figure 24. Crypt Parasites by Infection Status and by Days pi

The average number of parasites counted in the loop section of every location in each infected piglet through the course of infection in A.) individual infected piglets and B.) Isospora suis infected group.



A



B

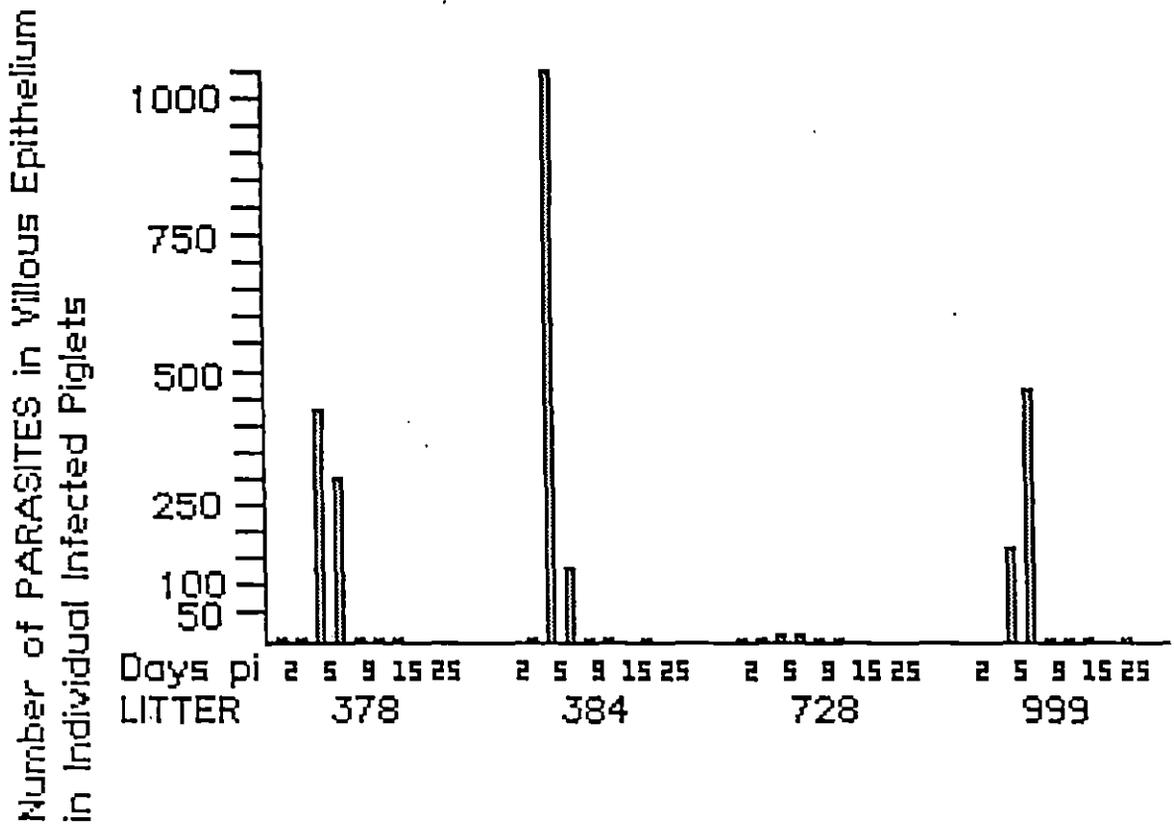


Figure 25. Enumeration of Parasite Numbers per Litter

The average total parasite count by infected litter at days pi.

Figure 26. Enumeration of Parasite Numbers by Location

Percent of total parasite count by location at days pi.

Location A = Duodenum, Location B = Jejunum-100, Location C = Jejunum-200,
Location D = Lower-Jejunum, Location E = Ileum-15, Location F = Ileum-5,
Location G = Cecum, Location H = Proximal colon, Location I = Spiral colon

Figure 27. Enumeration of Parasitic Stages

Percent of total parasite count by stage of life cycle at days pi.

Stage A = Asexual, Stage F = Macrogametocyte, Stage M = Microgametocyte,
Stage O = Oocyst, Stage U = Unidentified.

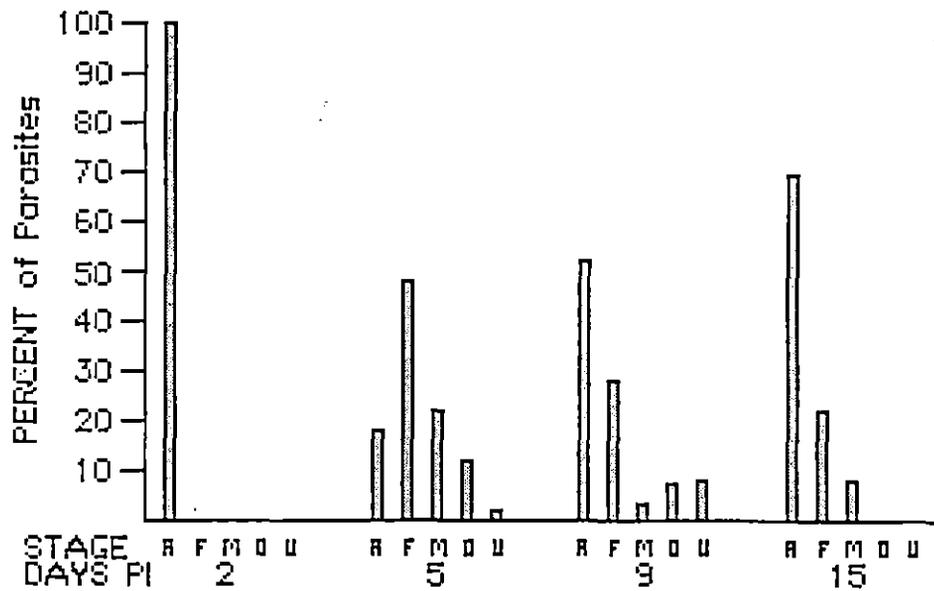
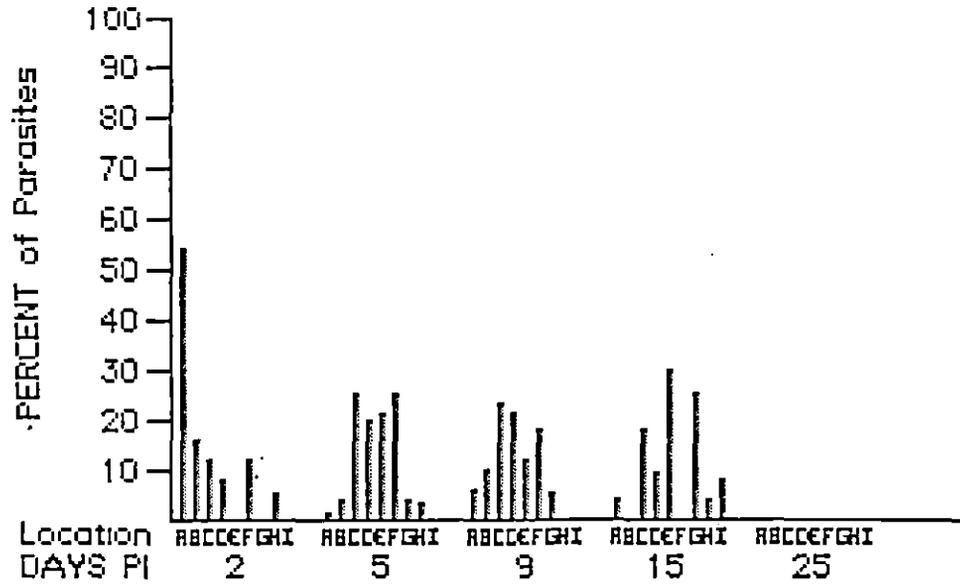
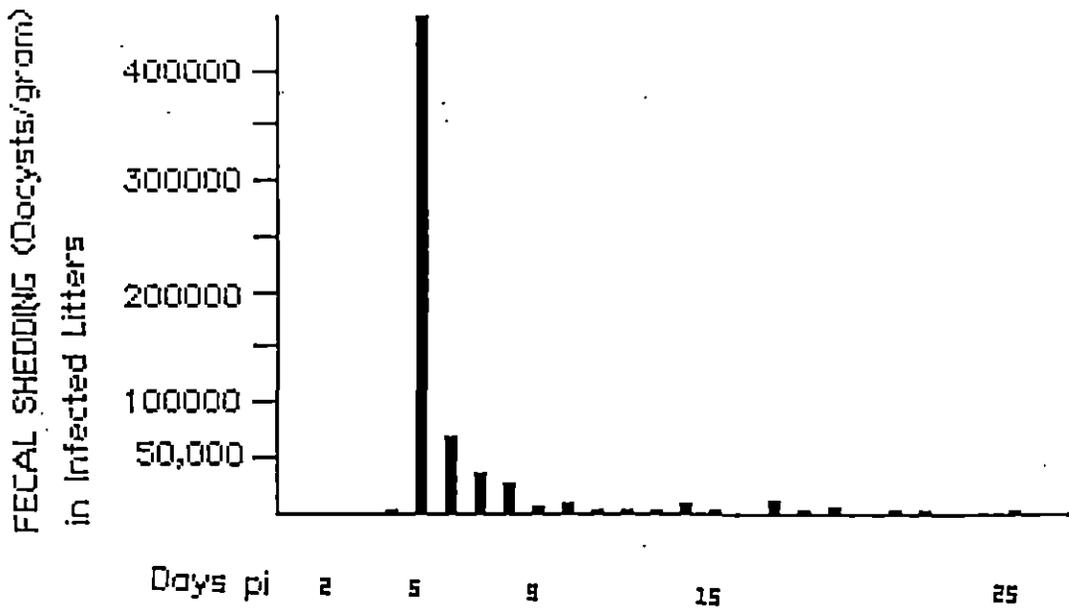
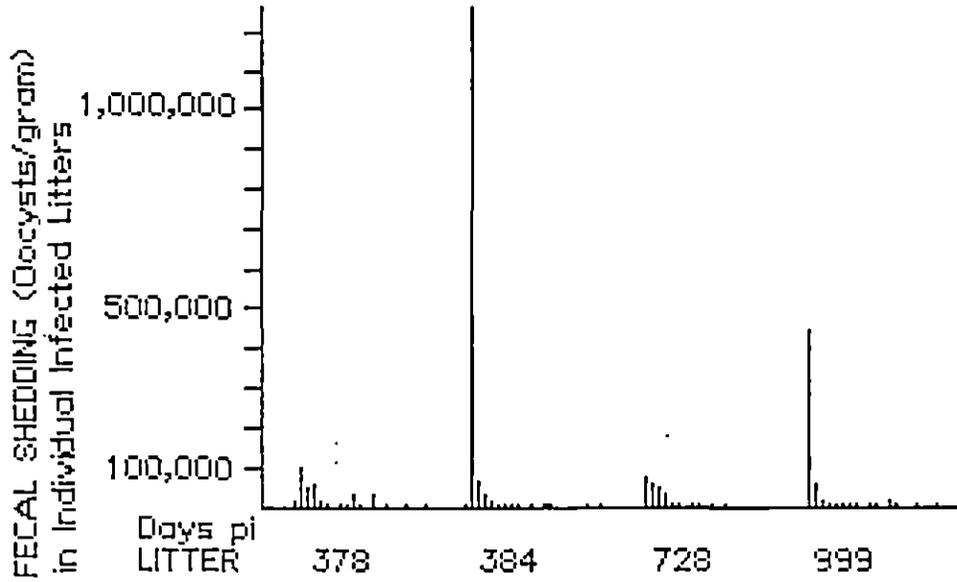


Figure 28. Individual Infected Piglet Fecal Oocyst Shedding.

The number of Isospora suis oocysts per gram of washed fecal sediment of individual infected piglets at the time of necropsy at days pi.

Figure 29. Infected Litter Fecal Oocyst Shedding

The number of Isospora suis oocysts per gram of washed fecal sediment of infected litters through the course of infection.



DISCUSSION

A dose of 70,000 sporulated Isospora suis oocysts produced clinical signs and histologic lesions in baby pigs similar to those reported in both field and experimental conditions. The time of onset of diarrhea was remarkably consistent at 3 days pi. Contrary to previous reports, at 3-5 days pi vomiting was frequently observed being nearly as predictable as the diarrhea. During the second and third days of clinical signs (4-5 days pi) the pigs appeared to be in abdominal discomfort. Generally, the pigs began looking better just as the vomiting stopped. The cause of the vomiting was most likely due to inflammation incited by the parasite infection in the upper small intestine, especially in the duodenum. The duodenum has been called the organ of nausea (Strombeck, 1979). Even at 2 days pi changes were apparent within the duodenum. There was a loss of villous epithelial cells with mild cuboidal changes at the tips of the villi. There was a mild decrease in numbers of villous goblet cells and a moderate increase in the number of villous intraepithelial lymphocytes ($p=0.05$). The consistent occurrence of vomiting in this experiment might be explained by the bolus inoculation of parasites. In contrast to natural infections where piglets are more likely to consume sporulated oocysts at a gradual rate, the piglets in this experiment were inoculated in one bolus dose. The majority of the inoculum oocysts most likely excysted in the duodenum within a short period of time thus assaulting the duodenal mucosa with an abundance of oocyst contents and sporozoites. The upper small intestine may be particularly inflamed in this experimental induction of disease.

Another factor which might have caused the vomiting is interference with normal gastric emptying. In both colibacillosis and transmissible gastroenteritis it has been shown that gastric stasis precedes the onset of diarrhea. Reports on these diseases often note that at post mortem the animals have full stomachs (Leman et al., 1986). Distention of the stomach is a recognized stimulus for emesis. However, in this study the animals in the infected groups at 2 days pi did not appear to have more distended stomachs than did the control pigs. The fullness of the stomachs was merely interpreted as evidence to the fact that the pigs were eating. On 5 day pi, 4/8 of the infected pigs had empty stomachs. This seemed to have one of two explanations. Either the pigs had just emptied their stomachs by vomiting or the pigs were anorexic. Due to the degree of discomfort and malaise observed, the latter of the two explanations was favored. The irritability of the pigs was most likely due to discomfort and a general feeling of malaise. The discomfort may be associated with the intestinal damage, inflammatory response and local intestinal distension.

The cause of the diarrhea is ascribed primarily to a maldigestion/malabsorption syndrome as evidenced by the villous epithelial damage and the severe villous atrophy and fusion. The loss of mature enterocytes especially at the upper half of the villi would result in the loss of the brush border enzymes. These enzymes are responsible for much of the disaccharide and some of the peptide digestion. The severe villous atrophy and fusion lead to a dramatic reduction in surface area available for either digestion or absorption of water, electrolytes or nutrients.

Complicating the loss of fluid further is the possibility of transudation and exudation of fluids and proteins to the lumen of the gut. As the tight junctions between columnar epithelial cells deteriorate, and as cells become necrotic and slough from the villi, the basement membrane is exposed, allowing protein leakage from capillary beds. The acute inflammation could result in exudation.

In this experiment, histologic examination of the large intestine shows that there were few significant large intestinal lesions. There was a significant ($p=0.05$) yet mild decrease in goblet cell numbers within the crypts of the large intestine at 5 days pi and an equally significant increase in goblet cell numbers by 9 to 25 days pi. Explanations for the severe diarrhea seen in these pigs may include a decrease in the ability of the colon to absorb fluids. Normally the colon has a large capacity for fluid absorption (Argenzio, 1978). Diarrhea results when these capabilities are surpassed by the fluid load presented from the small intestine. According to one theory the maldigestion present in the small intestine results in the hydrolysis of disaccharides by the bacterial gut flora to either monosaccharides or short chain organic acids. Because these short chain organic acids do not readily diffuse through the colonic epithelium an osmotic diarrhea develops. A second theory suggests that the maldigestion in the small intestine leads to hydroxylation of unsaturated fatty acids by bacteria to form molecules similar in structure to ricinoleic acid. Since the latter is a known cathartic (castor oil), steatorrhea results (Argenzio, 1978). Steatorrhea has been reported in

Isospora suis infected pigs (Nilsson et al., 1984). Our results of pasty diarrhea also suggest a steatorrheic component. A third theory suggests that bile acids, normally reabsorbed by the ileum, are presented to the colon in high amounts due to impaired ileal function. These bile acids can in themselves alter the colonic capabilities of reabsorbing water (Argenzio, 1978).

Mortality rates as well as severity of lesions vary according to 1) the number of ingested sporulated oocysts; 2) the stage of parasite life cycle; 3) the age and immune status of the pig; and 4) the presence of concurrent infection. The number of oocysts used to inoculate pigs is a major consideration in comparisons to other studies. A dose of 70,000 sporulated oocysts is not large and yet remarkable clinical signs as well as histologic lesions were produced: vomiting, diarrhea, death, severe villous atrophy, moderate villous fusion, focal necrosis, sloughing of necrotic epithelial cells, squamous and cuboidal metaplasia, loss in numbers of goblet cells, increases in numbers of intraepithelial lymphocytes and neutrophils, and increases in mitotic rates. The mortality rate was 9.3%, and yet no grossly visible fibrinonecrotic membranes were seen.

The mortality of baby pigs with diarrhea is most often due to severe dehydration and metabolic imbalances, primarily a metabolic acidosis and hyperkalemia both leading to cardiac arrhythmias and ultimately cardiac arrest (Bywater, 1981). Additional factors include sludging of blood cells, hypoglycemia, hypothermia, the development of disseminated

intravascular coagulation (DIC) and/or concurrent secondary infections. Bacterial colonization of damaged intestinal epithelium may provide a port of entry for bacteria to gain access to the blood circulation. The ensuing bacteremia may develop into a terminal septicemia.

Villous epithelial changes, villous atrophy and villous fusion were all most severe at 5 days pi. At 9 days pi, although changes such as sloughing epithelium, focal necrosis, villous atrophy and villous fusion were still present, the recovering villi were covered by more columnar epithelium. The villi were longer than those seen at 5 days pi and goblet cells were reappearing on the villi. By 15 days pi recovery was more apparent with very little cuboidal epithelium or focal necrosis evident; that which was present was localized primarily to the lower-jejunum. Epithelial abnormalities were generally confined to the villous tips. The tips of villi were the last to recover columnar epithelium because the replacement cells are produced in the crypts and migrate up the villi. Villous fusion remained a prominent lesion. The villi themselves were longer than those seen at 9 days pi ($p=0.05$). At 25 days pi there was little villous epithelial change. The villi were back to control lengths but villous fusion was evident, especially in the lower-jejunum section. At 35 days pi few histologic changes were noted although villous fusion was still apparent in the lower-jejunum. The persistence of villous fusion may help explain the reduced weight gains by pigs that have recovered from Isospora suis infection. If this fusion is permanent the resulting loss of villous surface area available for digestion and absorption could be

responsible for decreased nutritional uptake and subsequent weight gains.

The differences in mitotic index between control and infected groups in this study are interesting. The infection and loss of villous epithelium may have stimulated the crypts to respond with an increase in mitotic numbers. This increase was first noted at 5 days pi ($p=0.05$) and peaked at 9 days pi ($p=0.05$), lagging behind the peak damage to the epithelium by a few days. The mitotic index was maintained well above control levels through 15 days pi ($p=0.05$). At 25 days pi the rate returned to normal and at the jejunum-200 section actually was significantly ($p=0.05$) below the control level. This dip may be due to overexertion of the crypts in an attempt at repair resulting in either exhaustion or negative feedback.

Another explanation for the increase in intestinal mitotic rates is that the parasite may induce an immunologic response resulting in an increase in intraepithelial lymphocytes (T cells). These T-cells may secrete an enteropathic leukokine which acts directly on the crypt cell to increase the mitotic rate. This theory is supported by reports of parasites, primarily Giardia, stimulating an increase in mitotic rates without causing epithelial changes or villous atrophy (MacDonald and Ferguson, 1978; Gillon et al., 1982; Olveda et al., 1982; Remigo et al., 1982). Isospora suis may affect the mitotic rate by this mechanism. However, the parasite is destructive enough that this theory may get lost in the final analysis. It will be difficult to separate the stimuli inducing the increased mitotic rate. In this study the mitotic rate peaks at 9 days pi, lagging behind both the peak in epithelial damage and the

increase in the number of intraepithelial lymphocytes within the crypts by a few days.

Goblet cell numbers decreased as the goblet cells were stimulated to release their mucus in the course of the infection and as epithelial numbers dropped and villous lengths shortened. Numbers of goblet cells within crypts changed very little. There was a significant ($p=0.05$) decrease in crypt goblet numbers in the duodenum and ileum at 5 days pi and in the duodenum at 9 days pi. A small increase was seen first in the lower-jejunum and ileum-15 sections at 9 days pi and then throughout the intestine at 15 days pi. Some of this increase may be attributed to the increase in the number of cells within a crypt solely as a function of crypt depth.

At 2 days pi there was an increase in the number of intraepithelial lymphocytes in the villi of the duodenum ($p=0.05$), probably due to stimulation from the presence of the parasite. At 5 days pi, although the intraepithelial lymphocytes within the crypts were significantly increased ($p=0.05$), the number of villous intraepithelial lymphocytes was decreased throughout the small intestine ($p=0.05$) in the mid-jejunal and ileal sections. The increase in crypt intraepithelial lymphocytes is most likely associated with an immunologic response to the presence of the parasite. The intraepithelial lymphocytes are thought to be T-lymphocytes involved in cell mediated immunity (Ferguson, 1977). The decrease in villous intraepithelial lymphocytes at 5 days pi is most likely associated with the loss of epithelial cells and villous surface area. At 15 days pi

there was a significant ($p=0.05$) and marked increase of villous intraepithelial lymphocytes.

The numbers of intraepithelial neutrophils in both villous and dome epithelium were increased as early as 2 days pi, especially in the domes of ileum-5 sections. The domes seemed to be particularly sensitized to respond to the presence of the parasites. The numbers of villous intraepithelial neutrophils remained significantly ($p=0.05$) elevated throughout most of the middle and posterior sections of the small intestine out to 15 days pi. The most severe infiltrations were seen at 5 days pi in association with the greatest number of parasites and the most extensive intestinal damage.

The lamina propria contained increased numbers of neutrophils in the duodenum and ileum-15 sections ($p=0.05$) at 2 days pi. There were significant ($p=0.05$) increases of neutrophils in the jejunum-200 and lower-jejunum sections at 5 days pi. At 9 days pi there was a significant ($p=0.05$) and marked increase in neutrophil numbers within the lamina propria throughout the small intestine, especially apparent in the jejunal and ileal sections.

Many eosinophils were seen in the lamina propria of both control and infected piglets. At 15 days pi there was a significant ($p=0.05$) decrease in eosinophil numbers in the infected piglets at all examined locations of small intestine. This decrease was maintained out to 25 days pi ($p=0.05$) in the lower-jejunum and ileum-5 sections.

There was an increase in plasma cell infiltrations of the lamina

propria of the small intestine, especially in the ileal sections by 15 days pi. At 25 days pi the difference between control and infected piglet plasma cell numbers seemed to lessen. By 35 days pi no difference was noted.

In infected pigs at 5 days pi, the villi were significantly ($p=0.05$) shorter, the crypts were significantly ($p=0.05$) deeper and the villous/crypt ratio was significantly ($p=0.05$) decreased in comparison to controls. At 9 days pi the villi remained shorter ($p=0.05$) and the crypts remained deeper ($p=0.05$). The most significant differences between infected and control groups were seen in the lower-jejunal and ileal sections. By 15 days pi the villi were shorter ($p=0.05$) in all examined locations; the crypts were significantly deeper ($p=0.05$) only in the jejunal sections. At 25 days pi there was little difference noted between the control and infected pigs' villous and crypt lengths except for a decrease ($p=0.05$) in crypt depth in the infected piglets' duodenal sections.

No differences were noted between infected pigs and control pigs as to the mitotic rate, the number of intraepithelial lymphocytes, or the number of intraepithelial neutrophils in the large intestinal sections. There is a dramatic line drawn by the data between the effects of the parasite on the small intestine and the effects of the parasite on the large intestine. This is likely due to the fact that few parasites infect large intestinal epithelial cells.

It was noted that severe inflammatory infiltrates and villous

epithelial changes were seen frequently without an association with parasitized epithelial cells. It has been suggested that there may be other components of the parasite that cause damage to epithelial cells and inflammation. The oocyst contents, released upon excystation, may be toxic to epithelial cells. The byproducts of each meront, released into the lumen as it ruptures the cell may be noxious to nearby epithelial cells. There are reports of coccidial extracts being lethal to epithelial cells (McKenzie et al., 1985).

The parasite distribution within the intestines of the pigs in this study was similar to that found in most other reports (Cesario, 1980; Lindsay et al., 1980; Matuschka and Heydorn, 1980; Harleman, 1982). At 2 days pi the majority of parasites were seen in the duodenum with progressively declining numbers seen in the jejunum-100, jejunum-200, lower-jejunum, ileum-5 and proximal colon. At 5 and 9 days pi the distribution centered around the mid-jejunum and ileum. At 15 days pi parasites could be seen in sections from the duodenum to spiral colon. Many parasites were centered around the mid-jejunum to ileum but there was another peak in the cecum and a smaller peak in the spiral colon. This finding supports other reports that the parasite distribution shifts toward the large intestine over time.

Previous reports also note that the distribution of parasites shifts from the villous to the crypt epithelium over time (Harleman, 1982; Harleman and Meyer, 1984). This study supports those findings. At 5 days pi 99.62% of the parasites were within villous epithelium with only 0.4%

within crypts. At 15 days pi 74.0% were in crypt epithelium. Harleman (1982) regards this shift as an indication that there may be extraintestinal stages and that the emergence of the parasite in crypt epithelium is actually the parasite returning to the gut epithelium from deeper tissues. Yet it may be that the crypt epithelium is simply the more susceptible site of infection at 15 days pi. It should be pointed out that although the percent of infected epithelial cells in crypts was high at 15 days pi the absolute numbers were well below the number of parasites seen in crypts at either day 5 or day 9 pi.

The localization of the parasites within the intestine is thought to be due to cell surface receptor differences found along the intestinal tract (Soulsby, 1976). Time differences associated with location may be due to changes in these receptors as cell damage and renewal occur, to local immunologic differences or to the directional flow of the intestinal contents.

The Brunner glands of the duodenum were carefully examined for parasitized cells. In contrast to Harleman's findings (1984), no parasites were found in these glands.

In this study different types of meronts were not identified. In 5 u histologic sections cells were cut in various planes such that entire meronts were not readily identifiable. It seemed unreliable to use measurements of the organisms in H & E sections in order to define their type; therefore, no attempt was made to make any distinction between the asexual forms of the parasite.

Distribution of parasites within life cycle stages were very similar to those reported elsewhere. Only asexual stages were seen at 2 days pi. No pigs were killed at 3 or 4 days pi. The first sexual stages were seen at 5 days pi. Percentages of stages at 5 days pi were similar to those given by Lindsay et al. (1980). Both asexual and sexual stages were seen at 5, 9 and 15 days pi. A large asexual population was maintained on both 9 and 15 days pi with a fairly steady distribution within intestinal locations.

Domes were initially infected by Isospora suis at 2 days pi and more frequently at 5 days pi. The distribution of the stages of the parasite was nearly identical to that seen in the villous epithelium. The infection was associated with epithelial changes as described for the villi: Cuboidal and squamous metaplasia, focal necrosis, microulcerations and a neutrophil infiltration of both the lamina propria and the intraepithelial spaces. All of the above changes were most severe at 5 days pi but occurred at 2 days pi as well as 9 days pi. At both 5 and 9 days pi fusion between dome epithelium and adjacent villi was noted.

The infection of these domes may have important implications. The domes are a primary site of developing intestinal immunity; they specialize in the uptake and processing of macromolecular antigens. They are covered by absorptive cells, goblet cells and a specialized epithelial cell termed "M cell". Dome areas may be associated with the production of immunocompetent B cells (Chu and Liu, 1984). The M cells are associated with lymphocytes which may react with gut lumen antigens at the level of

the dome epithelium. This specialized epithelium may facilitate the recognition of antigen (Anderson, 1977; Chu et al., 1979; Torres-Medina, 1981; Chu and Liu, 1984). Damage to these areas implies a loss of immunologic capabilities against gastrointestinal pathogens thus making the piglets particularly susceptible to secondary infections. The fusion of domes to neighboring villi implies a loss of surface area available for contacting intestinal antigens. Loss of epithelium or increased pore size in dome cells may also lead to the uptake of large macromolecular antigens which could result in harmful dietary allergies (Patterson, 1972).

In this study a few parasites, often identified as macrogametes, were found subepithelially and within the lamina propria most frequently of domes but also of villi. Many of these parasites were so close to the epithelial layer that it was difficult to discern whether the parasite was truly subepithelial or simply a swollen distorted parasitized epithelial cell bulging into the lamina propria with the section cut in such a manner as to accentuate the distortion of the epithelial cell. However, some of these parasites appeared to be actually below the epithelial layer (Photographs 16 and 17). This has not been previously reported in Isospora suis infections. This has implications with regard to our knowledge of the life cycle of Isospora suis. Perhaps these subepithelial parasites indicate that there is a port of entry for Isospora suis to travel to extraintestinal sites. The parasites may be within lymphocytes or macrophages and transported as reported for various Eimeria species (Soulsby, 1982). The parasites may gain entry, as reported in Giardia

infections (Olveda et al., 1982), via breaks in the epithelial lining, particularly that of dome areas of the intestine. No parasites were seen deeper in the intestinal wall.

Differences in microscopic lesions as well as in clinical signs were observed between infected litters. Histologic lesions seemed to correlate with parasite numbers seen in histologic sections. No correlation could be made between any histological abnormality and a pig's output of oocysts/gram of feces on the day of necropsy. Piglets from litter 384 (L384) in general were the most severely affected pigs with L378 piglets having nearly as severe intestinal changes. Clinical signs were most severe in these litters. All dead pigs come from these two litters with a mortality rate amongst themselves of 17.4%. Histologic lesions were most severe at 5 days pi in litter 384. At 15 days pi L384 pigs also had the most obvious lesions when compared to age-matched pigs from other infected litters. This corresponds well to the comparison of parasite intestinal stages present in each pig. At both 5 and 15 days pi the intestinal parasite counts in pigs of L384 were higher than age-matched infected pigs. At 9 days pi, when the number of parasites seen were much greater in pigs from L378 than in those from L384, the severity of histologic lesions followed with greater abnormalities being noted in pigs from litter 378.

Piglets from L728 were consistently the least affected. Clinically the pigs may have been slightly less affected although vomiting, abdominal discomfort and diarrhea were all observed. Piglets did not appear dehydrated. There were no mortalities. Lesions in all pigs were milder

than age-matched pigs from other infected litters. By percentage of total parasites seen on a particular day pi, piglets from L728 had the fewest number of parasites on all days except 2 days pi when L999 had two less parasites observed.

For individual pigs, the number of oocysts/gram of feces at the time of necropsy did not correlate with the severity of any of the histological abnormalities found. In contrast, average litter lesions did seem to follow the pattern of litter fecal oocyst shedding. The litters shedding high numbers of oocysts generally had the most severe histologic lesions.

The differences between infected litters might be explained by: 1) genetic differences; 2) differences in colostral and/or lactogenic immunity; 3) complications of concurrent infection; and 4) different potencies of inoculum. However, no such differences between the infected litters in this study were documented. Genetic differences in the litters of these crossbred sows are easy to imagine but would be difficult to evaluate. Lactogenic immunity may have promoted the differences seen between litters. Sows 999 and 378 did suffer from mastitis and/or hypogalactia. This may correspond to the presence or absence of the lactogenic immunity received by the piglets. How the colostral and lactogenic immune status of the piglets affected the outcome of the parasitic infection is not known. Maternal immunity is not thought to be protective in coccidial infections (Rose, 1972). Most immunologic studies on coccidia indicate that the cell mediated response is the more protective avenue of immunity. How much of this is passed to the piglet via colostrum

and milk and how this is altered by mastitis is unknown. Perhaps the colostral and lactogenic immunity become important in preventing secondary infections and complications (Rogers Brambell, 1970). Piglets from L999 and L378 could have received less protective immunity against other gastrointestinal pathogens thus upsetting the normal gut flora enough to allow the Isospora suis a stronger foothold. Another factor which could influence the differences seen between the litters of infected piglets is the inoculum itself. Although alterations of the inoculum were kept to a minimum, changes in the inoculum over time should be considered as a source of error. The sporulated oocysts were obtained from the same pool of inoculum. Because each litter was infected on different calendar days, the inoculum for each litter was adjusted to contain the same concentration of viable sporulated oocysts. Litter 728 was the last litter infected and the least affected litter. However, a litter of piglets not included in this study was infected with the same pool of inoculum subsequent to the dosing of L728 and it was severely infected with Isospora suis. This result indicates that the pool of inoculum maintained its infectivity over time. Reviewing the histologic slides of L728 at 2 days pi, it is difficult to maintain that the infective dose was less than that of the other litters. The inflammation and reaction to the parasite inoculum was as severe if not the most severe of all the litters. For some reason, either the sporulated oocysts could excyst and produce quite a noxious reaction yet were physically or chemically incapable of infecting the epithelial cells, or the piglets of L728 reacted quickly to clear the parasitic infection and

recover from the damage much more rapidly than the other infected piglets. Perhaps there was a significant genetic or immunologic resistance in this litter.

SUMMARY

Four litters of piglets reared with their dams were orally inoculated with 70,000 sporulated oocysts of Isospora suis at 36 hours of age. Four other litters reared with their dams were used as age-matched uninfected controls. One or two piglets from each group were euthanized at 3, 6, 10, 16, 26 and 36 days of age.

No control animals were clinically ill; they did not pass any Isospora suis oocysts in their feces and they did not have any notable histologic abnormalities within their intestinal tract.

In all infected litters vomiting was observed at 3 to 5 days pi. At 3 to 12 days pi the infected litters had diarrhea. At 6 to 11 days pi four of the 43 infected animals died.

The histologic lesions in the infected piglets were systematically documented and compared to those in age-matched controls. In general, infection with Isospora suis sporulated oocysts caused significant changes in all examined parameters. There were villous epithelial degenerative changes, villous atrophy, crypt hyperplasia, and villous fusion. The number of villous goblet cells decreased; the number of crypt goblet cells increased. The number of both crypt and villous intraepithelial lymphocytes increased. The number of intraepithelial neutrophils increased. The neutrophilic infiltrations of the lamina propria increased while the eosinophilic infiltration decreased. The lesions were restricted to the small intestine, with the most severe changes centering around the posterior jejunum to anterior ileum areas. Considering the number of

parasites infecting epithelial cells and the severity of the histologic lesions it was remarkable that no fibrinonecrotic membrane was observed in any of the piglets.

The striking lesions found associated with the domes of the small intestine, evident as early as 2 days pi, led to speculation on the importance of the domes to the Isospora suis infection. At 2 days pi there was a marked infiltration of neutrophils within the dome areas. A few domes were infected with Isospora suis merozoites. By 5 days pi the infection rate of the domes was high. Later, fusion between domes and villi was noted. These lesions may all have immunologic repercussions for the Isospora suis-infected pig.

The parasite distribution along the length of the intestinal tract during the course of infection was in agreement with previous reports. At 2 days pi the majority of the parasites were seen in the duodenum. At 5 days pi the peak number of parasites was seen in the posterior jejunum. By 15 days pi most of the parasites were in the large intestine. The parasites seemed to move in a caudal direction over time. Concurring with Harleman (1982), this study found that the proportion of parasites found within crypt epithelium increased over time, a finding Harleman used to support the possibility of extraintestinal stages of Isospora suis in the pig. Another finding in this study may give further credence to the theory of extraintestinal stages of Isospora suis: Parasites were found within host cells located subepithelially usually within the lamina propria of the domes.

The villous fusion documented in this study is considered an important finding in that it may help to explain the long term effects of Isospora suis infections in pigs such as poor feed efficiency and poor weight gains. At both 25 and 35 days pi most other histologic lesions of the Isospora suis infection had disappeared. The intestinal tract appeared to have recovered. The only outstanding and significant lesion remaining was villous fusion. This appears to be a long term lesion. It is not known whether villous fusion may in fact be a permanent change. The resulting loss of villous surface area could be the basis for poor doing of "recovered" Isospora suis infected piglets.

This study graphically illustrates that the parasite Isospora suis is capable of causing drastic and devastating damage to the intestinal tract of the baby pig.

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