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PATHOLOGY OF NATURAL ISOSPOROSIS IN NURSING PIGLETS

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Abstract. In piglets suffering from natural coccidiosis, post-mortem examination showed that pathological changes induced by *Isospora suis* were evident from day 7 to day 14 of life, and particularly, by days 9 and 10. Macroscopically, the changes were manifest as enteritis varying from catarrhal to pseudomembraneous form. Microscopically, they consisted of more or less extensive atrophy of villi whose apical parts were necrotic, of metaplasia and erosion of epithelium. With the exception of duodenum and the adjoining sector of jejunum, the alterations were manifest along the entire small gut though intensity of lesions and incidence of endogenous stages of *Isospora suis* varied from sector to sector of the intestine. Predilected was a portion limited approximately by 50 and 140 cm cranially from ostium ileocecale, viz. the caudal sector of central jejunum and the cranial sector of the caudal jejunum. Within this area, lesions were more severe and frequent than in sectors situated cranially and caudally of it. The predilection persisted even in case of concurrent adenovirosis. The lesions contained meronts and gamonts at the same time though gamonts predominated. Advanced merogony and gametogony resulted in distinct displacement of cell nuclei and in cell walls bulging into the inner diameter of the gut. We assume that endogenous stages of *Isospora* penetrate the submucosis via the narrow opening at the orifice of lymph follicle; such was the case with gamonts and oocysts detected in activated lymph tissue of Peyer's patches.

Recent research work definitely classified *Isospora suis* as a pathogen producing diarrhea in suckling piglets. Pathology of natural isosporosis in piglets was studied by Eustis and Nelson (1981), Sanford and Josephson (1981), Robinson and Morin (1982), Sandford (1983), and Morin et al. (1983). The macroscopical picture they encountered in the intestine of naturally infected animals was variable and ranged from catarrhal purulent inflammation to necrotic enteritis. Incidentally, fibrino-necrotic pseudomembranes were registered on the inner surface of the small intestine.

Histological examination revealed villous atrophy, necrosis and desquamation of epithelial cells, and, hyperplasia of the epithelium of crypts (Eustis and Nelson 1981, Sandford and Josephson 1981, Robinson and Morin 1982, Sandford 1983, Morin et al. 1983). Severe affection occasionally resulted in a complete loss of villi and in a cumulation of neutrophiles in the propria (Eustis and Nelson 1981). At the apices of villi were deposits of a homogenous eosinophilic mass which after special staining displayed rather features of collagen than of fibrin or amyloid (Eustis and Nelson 1981).

The objective of our study was to appreciate timing and character of pathologic lesions induced by *Isospora suis* to piglets reared in Czechoslovak large-scale piggeries, and, to compare results with literature data.

MATERIALS AND METHODS

Our set comprised 54 piglets (*Sus scrofa* var. *domestica*) examined instantly after killing. Among them, 48 were killed at the age of 7 to 14 days (1 × 7 d., 3 × 8 d., 7 × 9 d., 7 × 10 d., 12 × 12 d., 5 × 13 d., and 6 × 14 d.). In addition to it, 6 piglets were examined at the age of 17 and 18 days.

All of them were scouring at the time of examination or shortly before. The piglets originated from 8 large-scale piggeries with different management.

Pathological studies were carried out in piglets whose contained oocysts of *Isospora suis*. Feces were examined by flotation employing Sheather's solution (500 g sugar, 320 ml water, 6.5 g phenol). Bacteriological examinations were performed in order to exclude specific pathogens. In two breeds, absence of coronaviruses, rotaviruses and enteropathogenic strains of *E. coli* was confirmed by electronmicroscopy. In one breed, a concurrent infection by adenoviruses was ascertained as reported elsewhere (Vitovec et al. 1985). In another breed, *Isospora suis* was the only pathogen producing diarrhea.

Samples were collected immediately after killing. In the first place we took a specimen of ileum from a spot distant not more than 5 cm from ostium ileocaecale. More specimens were collected at 15 cm, 50 cm, and then at each point distant successively 30 cm from ostium ileocaecale so that the last one was taken from the duodenum. In the large intestine, we usually took one specimen from the apex or corpus of the caecum, one or two from the colon, and one from the rectum. Specimens for histology were also collected from the liver, kidneys, spleen, lung, brain, pancreas, regional mesenterial lymph nodes, and eventually elsewhere. Histology revealed no pathological alterations or moderate changes usually of nonspecific character signaling activation of lymph tissue, minute haemorrhage or dystrophy. Since they appeared to have no effect on the endogenous cycle of the protozoa under study we refrained from describing them specifically.

As a part of post-mortem we took scrapings of mucosa from different portions of the gut, smeared them and stained by Giemsa to evaluate incidence of endogenous stages of coccidia as recommended by Stevenson and Andrews (1982) and Lindsay et al. (1983). Samples from the gut or other organs to be examined histologically were fixed in 10% neutral formol. The material was prepared by the conventional paraffin method. Sections were stained by haematoxilin and eosine. Azur-eosine stain employed to identify protozoa in the gut, alcian blue and PAS reaction to determine mucopolysaccharides. Semi-thin sections were prepared by means of ultramicrotomes LKB 3 and Tesla BS 490 A. We stained them by toluidine blue and also polychromatically according to Warmke and Sheu-Ling Janet Lee (1976).

RESULTS

Among the 54 piglets excreting oocysts of *Isospora suis* with feces, simultaneous presence of endogenous stages of the coccidium and of morphological changes in the intestine could be recorded in only 21 piglets. With the rest of piglets, there was either changes affecting primarily the middle and rear portions of intestine. In a number of them or the alterations remained too gentle to reach the level of intestinal catarrh. Among these 21 animals, one piglet was aged 8 days, 7 piglets 9 days, 6 piglets 10 days, 2 piglets 11 days, one piglet 13 days, and 4 piglets 14 days. Animals older than 14 days excreted no or very few oocysts, and lesions were moderate while sings of postinflammatory reparation predominated.

The macroscopically manifest consequences of natural isosporosis are inflammatory no relation between pathologic alternations and endogenous stages of *I. suis* within cases, conspicuously affected was the portion situated between 50 and 140 cm cranially from ostium ileocecale which included parts of the central and caudal jejunum. It was exclusively within this portion that we in two cases discovered pseudomembranes either freely in the gut or loosely adherent to hyperaemic and oedematous mucosa. The pseudomembranes were of soiled yellowish-gray colour. Corresponding lymph nodes were enlarged, hyperaemic, and oedematous.

Histological examinations of piglets aged 8—11 days indicated that villi were affected primarily. They showed various degrees of atrophy associated with changes in their apical epithelium. Some of the atrophic villi were enlarged at the base while the epithelium at the apex was eroded or the apex was covered by metaplastic pavement epithelium (Plate I, Figs. 1, 2). Sometimes the apex of necrotic villi was coated by pseudomembranes consisting of a fibrin network and a number of desquamation epithelium cells, regressive cell elements of inflammatory infiltration and numerous endogenous

stages of *I. suis* (Plate II, Figs. 1, 2, Plate III, Fig. 1). In areas of atrophic villi, intestinal crypts were mostly extended and littered by hyperplastic immature epithelium. The lamina propria of the apical part of villi was often intensively hyperaemic, edematous and imbued by blood extravasations. Mixed inflammatory infiltrations were only locally augmented, occasionally displaying a marked proportion of eosinophiles. Sporadically, the lamina propria at the apex of eroded villi included eosinophilic shapeless masses which were negative when stained by alcian blue, Gram or PAS. Exposed to trichromium staining, some parts of the necrotic mass acquired blue colouring while other a red one indicating so that they partly consisted of hyalined structureless protein material.

With this category of piglets (aged 8 to 11 days), the stroma of both atrophic and unaffected villi was covered by enterocytes whose cytoplasma was markedly bright, vacuolized, and voluminous. The stroma in these areas was thin and edematous, cells incidentally contained substances which reacted positively to alcian blue and PAS.

Endogenous stages of *Isospora suis* found within the described morphologic alterations were situated in parasitophorous vacuoles. Asexual stages were represented in the first place by spherical meronts often consisting of numerous sickle-shaped merozoites. Among sexual forms, we mostly identified spherical or ovoidal macrogamonts in different stages of maturity. Less often encountered were ovoidal or extended microgamonts bearing on their periphery bases for microgamets. Frequent were both immature and mature oocysts. In azur-eosine, the cytoplasma of development stages acquired a bright blue colour while nuclei were eosinophilic.

Endogenous stages of *Isospora suis* occupied the larger part of the intestine. They were absent in the duodenum and adjoining sectors of the cranial jejunum. The highest incidence was recorded within the sector from 50 cm to 140 cm cranially from ostium ileocecale, viz. in the caudal portion of the central and the cranial portion of the caudal jejunum.

Accordingly, the most severe lesions were located within this sector. As a rule, we identified both meronts and gamonts. The share of gamonts was larger but for two piglets of 9 days where from 100 cm to 150 cm cranially from ostium ileocaecale we identified exclusively meronts while sectors situated more cranialy or caudally were occupied predominantly by gamonts. The majority of endogenous stages of *I. suis* invaded the epithelium on the apical part of villi while few on their base and none on the epithelium of crypts. They were equally numerous in the absorption epithelium which included vacuoles and in nonvacuolated epithelium. They were easier to detect in the bright vacuolated absorption epithelium (Plate III, Figs. 2, 3).

Detecting endogenous stages of *Isospora suis* was extremely difficult in the caudal sector of central jejunum and the cranial sector of caudal jejunum in cases of vastly eroded epithelium and necrotic apical parts of villi in connection with pseudomembranes. Sporadic findings were registered merely in the wall of villi preserving residues of bright vacuolated epithelium. In such cases, numerous endogenous stages were present in the lesser destroyed sectors who cranially or caudally adjoined the area of necrosis and oocysts and gamonts invaded the activated lymph tissue of Peyer's patches (Plate III, Fig. 4).

The endogenous stages of *Isospora suis* were located in vacuoles within the cytoplasma of epithelial cells. In semi-thin sections they were to be detected below the nuclei of absorption cells. The nuclei were shifted towards the inner diameter of the intestine (Plate IV, Figs. 1, 2), and their displacement often caused a semispherical bulge of the cell wall.

Piglets aged 13—14 days presented from the pathological point of view an incongenial group. In one case of a piglet aged 14 days, we observed pseudomembraneous

inflammation as described earlier, in the rest of piglets endogenous stages of *I. suis* were located in indistinct morphological changes. In one piglet of 13 days and two piglets of 14 days, there were moderate atrophy of villi in central and caudal jejunum, and, the lamina propria mucosae contained increased amounts of mixed inflammatory infiltrations. Meronts and gamonts in epithelium were registered sporadically; in one case gamonts predominated. In another piglet aged 14 days, we detected a focus in the anterior jejunum which consisted of shortened intergrown villi covered by cylindrical epithelium. Meronts situated in bright vacuoles were registered only within this focus.

Endogenous stages of *I. suis* in smears prepared from mucosal scrapings matched those detected in histological preparations from the respective sector of gut.

DISCUSSION

According to Eustis and Nelson (1981), natural coccidiosis produced pathological changes in the intestine of piglets most frequently in animals aged 6 to 10 days. Sandford and Josephson (1981), Robinson and Morin (1982), Morin et al. (1983), and Sandford (1983) reported them between days 5 and 10, the highest incidence occuring from day 7 to day 10. Our trials placed alterations due to isosporosis into the period between days 7 and 14 with the maximum by days 9 and 10, thus two or three days prior to the highest number of oocysts in feces which Koudela et al. (1986) reported to be by day 12 of life. Beginning by day 13 we encountered in the intestine — bar one severe case — signs of restoration from atrophic alterations, and, endogenous stages of *Isospora suis* dissipated scarcely. The focus of meronts on intergrown atrophic villi in an otherwise intact intestine appeared to be a random finding.

Pathological alterations effected by natural coccidiosis in nursing piglets are variably situated. Eustis and Nelson (1981) reported them in jejunum and ileum, Sandford and Josephson (1981) in the major part of jejunum and ileum, Robinson and Morin (1982) and Morin et al. (1983) in the central and caudal jejunum and ileum. The usual macroscopic manifestation is a catarrh; fibrino-necrotic membranes in the major part of jejunum and ileum were reported in few cases. The histological picture is dominated by differently intensive atrophy of villi, metaplasia of epithelium, in severe cases by necrosis of the apical parts of villi and pseudomembranes (Eustis and Nelson 1981). We registered similar macroscopical and microscopical changes, and noticed their considerable variability in number and intensity within different sectors of jejunum and ileum. In our cases, most intensive lesions were confined to a sector distant 50 to 140 cm cranially from ostium ileocaecale. At the age of 7 to 14 days, this sector covers the caudal portion of central jejunum and the cranial portion of caudal jejunum. Portions situated cranially from this sector as well as the ileum were affected to a substantially lesser extent.

We detected endogenous stages of *Isospora* anywhere in the small gut except duodenum and the adjoining portion of jejunum. Their number also considerably varied in different portions of the bowel. As a rule, they were abundant in sites of severe and frequent lesions, viz. in the rear part of the central jejunum and the front part of the caudal jejunum. They were present equally in bright vacuolated epithelium and in epithelium with a homogenous cytoplasma. No endogenous stages were detected in epithelium of the large intestine. Sporadic endogenous stages of coccidia in the colon were reported by Sangster et al. (1976), Sandford and Josephson (1981), Robinson and Morin (1982), and Sandford (1983).

Likewise divergent are also reports concerning asexual and sexual stages in the intestine. Sandford and Josephson (1981) histologically identified mainly asexual stages. Morin et al. (1983) described them in piglets killed 48 hours after the onset of diarrhea. In our cases there were meronts and gamonts, but gamonts predominated. We recorded two unusual cases of piglets aged 9 days where in a sector between 100 and 150 cm cranially from ostium ileocecale, viz. in transitional portions of central and caudal jejunum, were exclusively asexual stages of *Isospora suis* while cranially and caudally from this sector gamonts predominated.

The axioma that most endogenous stages of *I. suis* occured in the caudal portion of central jejunum and the cranial portion of caudal jejunum stood unless intestinal epithelium was destroyed beyond a certain point. In cases with extensive necrosis and pseudomembranes, metaplasia and desquamation of absorption cells, detecting endogenous stages of *I. suis* directly in the intestine became sometimes extremely difficult. Merely in residual epithelium at the base of necrotic villi remained spherical macrogamonts with plainly visible eosinophilic nuclei. Numerous endogenous stages were then registered cranially and caudally from the affected sector where villi and epithelium were disintegrated to a minor extent. The implication is significant for practical diagnostics since endogenous stages of *I. suis* might be easily missed when concentrating merely on severely affected areas. The rare occurence of endogenous stages of coccidia in cases of necrotic enteritis was pointed out by Eustis and Nelson (1981).

It is obvious that the rear portion of central jejunum and the forward portion of caudal jejunum are predisposed to accommodate endogenous stages of *Isospora suis* no matter whether they act as the only etiological agent or concurrently with enteral adenoviruses or in cases where possible participation of other pathogens cannot be excluded.

The number of endogenous stages in smeared scrapings of the mucosa was always related to their number detected histologically in the same sector of the gut. Similar results were reported by Stevenson and Andrews (1982). The method of smears prepared from mucosal scrapings as described by Stevenson and Andrews (1982) turned out to be quick and reliable (Vítovec and Koudela 1986). Compared with histology the method is simple and renders diagnostic results within a very short period of time after killing the piglet.

A particularly unusual finding was the extraepithelial location of endogenous stages of *I. suis*. Only Lindsay et al. (1980) identified a macrogamont in the lamina propria of a piglet eight days post infectionem. In our material from spontaneous isosporosis, a cumulation of oocysts and gamonts in the submucosis was detected in the activated lymph tissue of Peyer's patches. They pressumably penetrated through the narrow opening in lamina muscularis at the orifice of the lymph follicle.

Lindsay et al. (1980) and Harleman and Meyer (1985) described that endogenous stages of coccidia are enclosed in a parasitoforic vacuole which is situated below the nucleus of the host cell. The situation of cell nucleus is hardly appreciable in histological sections while easily evaluable in semi-thin sections for ultramicroscopy. We stained semi-thin sections as described by Warmke and Sheu-Ling Janet Lee (1976). Lindsay et al. (1980) detected neither shift of nucleus nor bulging of the cell wall. Our results were different since the presence of endogenous stages of *Isospora suis* usually effected a distinct displacement of the nucleus towards the inner diameter of the intestine and consequently a semispheric bulge of the affected enterocyte. The phenomenon was observed primarily during the phase of advanced merogony and gametogony.

ПАТОЛОГИЯ СПОНТАННОГО КОКЦИДИОЗА МОЛОЧНЫХ ПОРОСЯТ

Й. Витовец и Б. Коудела

Резюме. Патологические изменения при спонтанном кокцидиозе молочных поросят встречались в возрастной группе от 7 до 14 дней после рождения — с наибольшей частотой у поросят возрастом в 9—10 дней. Макроскопически это были различные изменения диапазоном от катара по воспаление всей кишечной стенки. В гистологической картине преобладала в различной степени выраженная атрофия ворсинок кишечника, сопровождаемая в разной степени выраженным некрозом апикальных отделов ворсинок, метаплазией и эрозиями эпителия. За исключением двенадцатиперстной кишки и прилежащих отделов, тощей кишки эти патологические изменения встречались по всей длине тонкого кишечника, но эти изменения отличались наличием кокцидий разных стадий развития и масштабами патологических изменений в отдельных частях кишечника. С наибольшей частотой встречались патологические изменения и разные стадии развития кокцидий *Isospora suis* в области тонкого кишечника от 50 до 140 сантиметров, то есть в задней части средней кишки и передней части задней тощей кишки. В этой области наиболее часто встречались тяжелые патологические изменения; в областях расположенных более краниально и более каудально встречались более легкие патологические изменения и меньшее количество разных стадий развития кокцидий. Эта область оставалась местом наиболее частой локализации и в случае одновременного присутствия аденовирусов. В патологических очагах встречались одновременно стадии меронтов и гамонтов, но преобладали гамонты. В случае развитой мерогонии и гаметогонии наблюдалось выраженное распределение ядер и выпуклость мембраны пораженных клеток в просвет кишечника. Авторы придерживаются мнения, что разные стадии развития кокцидий могут проникать в субмукозу щелью в lamina muscularis mucosae в месте впадения лимфатического фоликула, подобно случаю обнаружения гамонтов и ооцист в активированной ткани Пейеровой бляшки.

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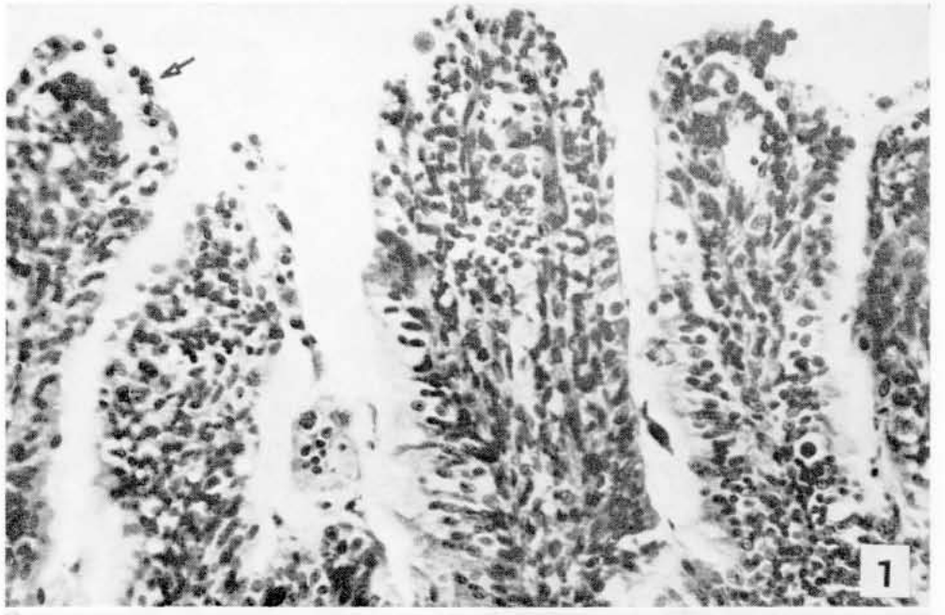
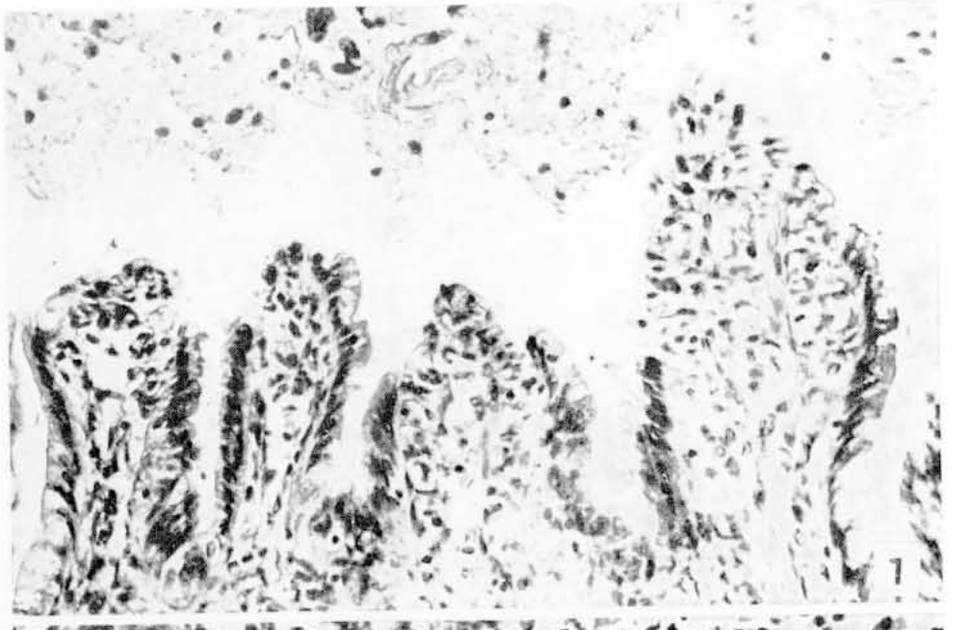


Fig. 1. Epithelial metaplasia (arrow) and erosions in apical parts of jejunal villi affected with endogenous stages of *I. suis*. (HE, 350×). **Fig. 2.** Villous atrophy in caudal jejunum of piglets infected with *I. suis*. (HE, 350×).



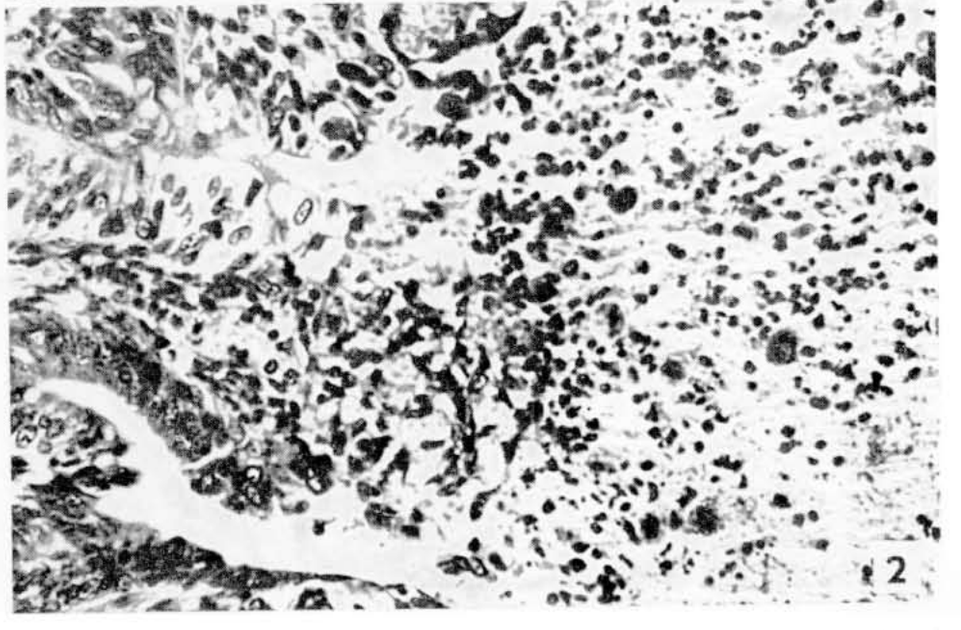


Fig. 1. Villous atrophy, epithelial erosions and necrotic masses in caudal jejunum in natural coccidiosis caused by *I. suis*. (HE, 350×). **Fig. 2.** Pseudomembranes and necroses of apical parts of jejunal villi in natural coccidiosis of suckling piglets caused by *I. suis*. (HE, 400×).

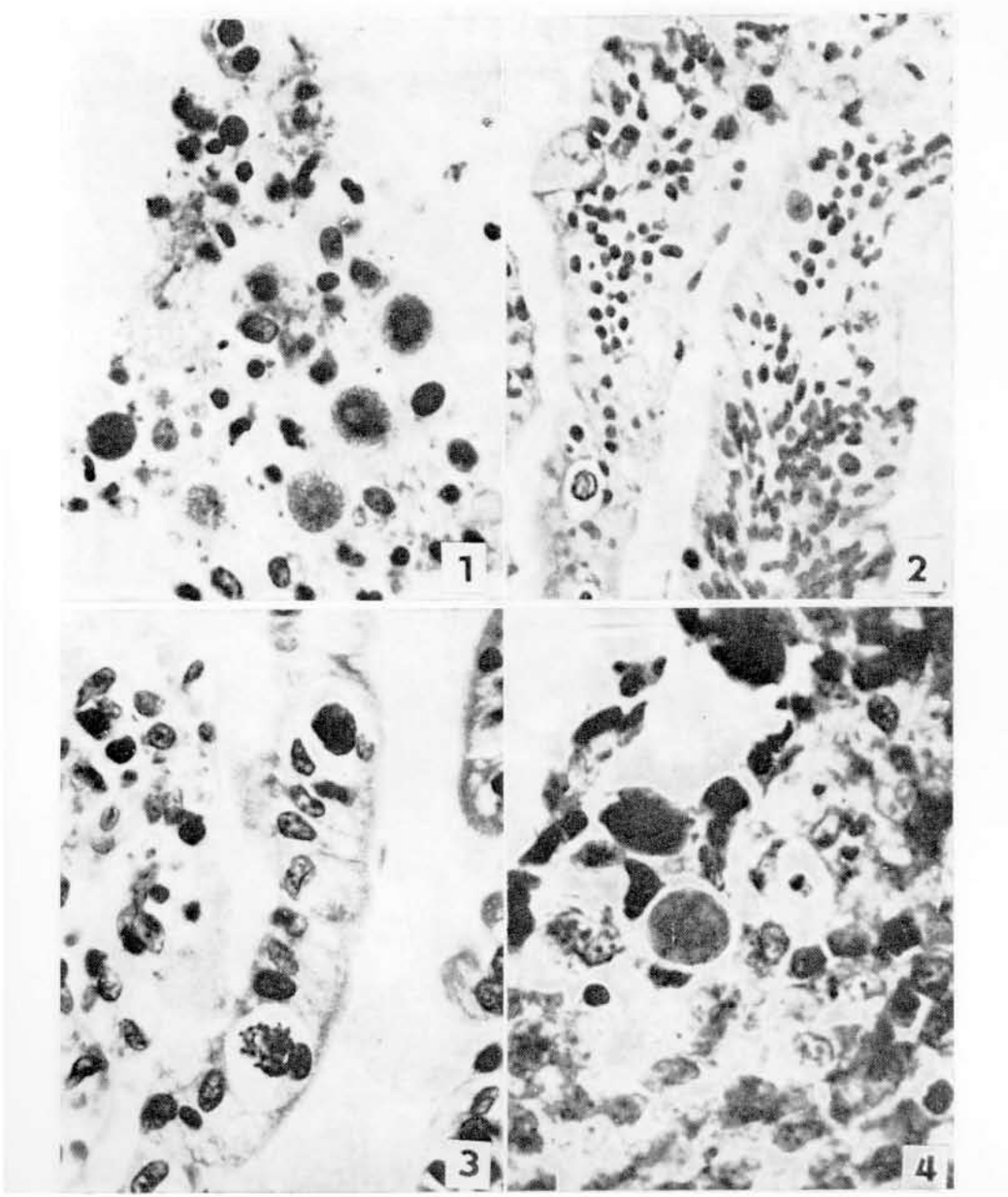


Fig. 1. Numerous endogenous stages of *I. suis* in pseudomembranes. (HE, 450×). **Fig. 2.** Vacuolated absorption epithelium in jejunum infected with gamonts and meronts of *I. suis*. (HE, 250×). **Fig. 3.** Multinucleate meront and microgamont in parasitophorous vacuole imbedded below the nuclei of enterocytes located in jejunal villi. (HE, 500×). **Fig. 4.** Endogenous stages of *I. suis* implanted in submucosal lymphatic tissue of Peyer's patches in ileum. (Azur-eosine, 700×).

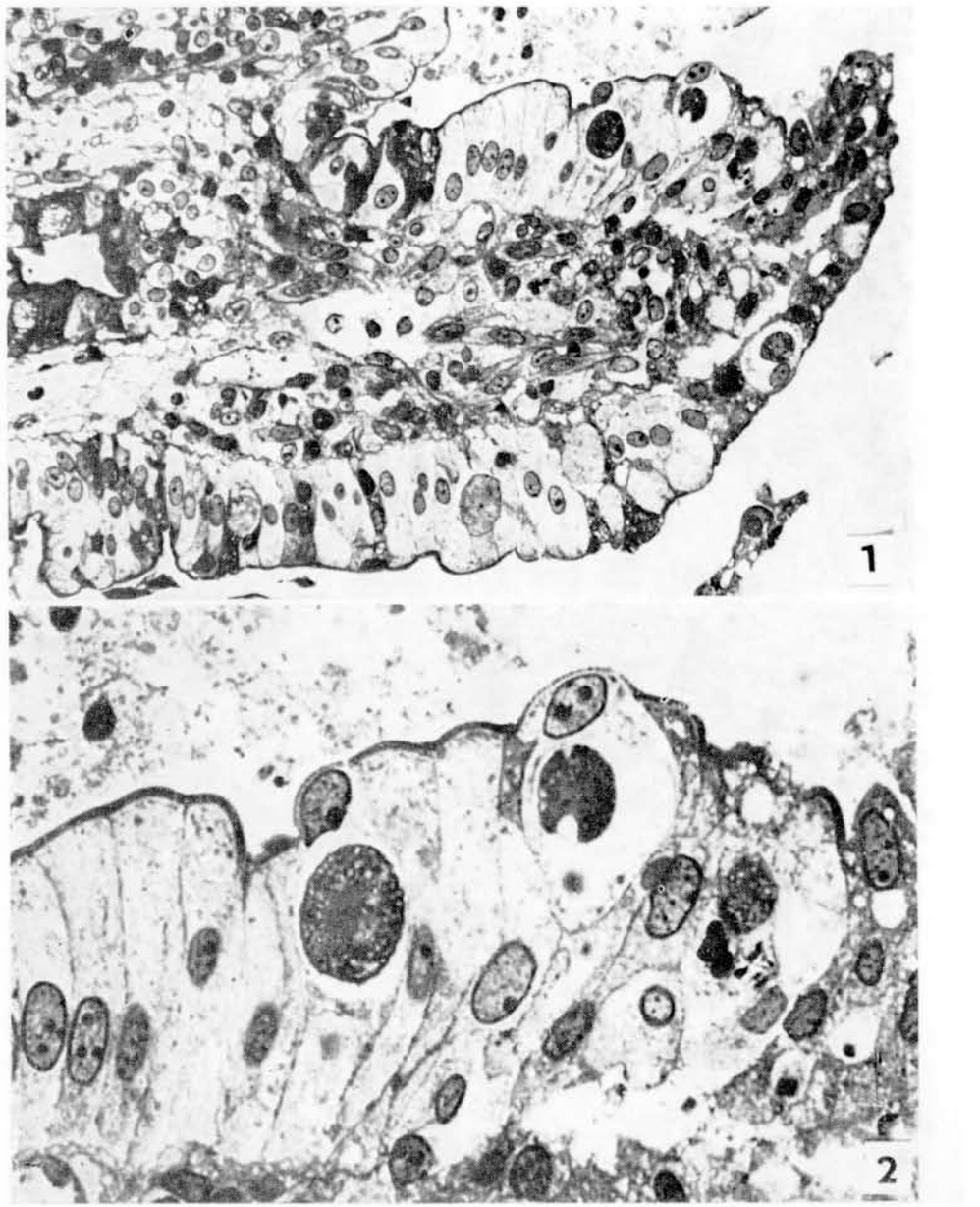


Fig. 1. Gamonts of *I. suis* within villous jejunal epithelium. Semi-thin section. (Warmke and Sheu-Ling Janet Lee, 500×). **Fig. 2.** Gamonts of *I. suis* within jejunal enterocytes. Displacement of the nucleus towards the inner diameter of the intestine and semispherical bulge of the cell wall. Semi-thin section. (Warmke and Sheu-Ling Janet Lee, 1.450×).