PATHOLOGY OF NATURAL ISOSPOROSIS IN NURSING PIGLETS

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Abstract. In piglets suffering from natural isosporiasis, 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 catarhal to pseudomembranous 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 60 and 140 cm cranially from oritum 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 adenoenteritis. The lesions contained necrotic foci and granulomas at the same time though granulomas 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 submucosa via the narrow opening at the orifice of lymph follicles; such was the case with granulomas and eosinophils 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 isosporiasis 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 catarhal purulent inflammation to necrotic enteritis. Incidentally, fibrino-necrotic pseudomembranes were registered on the inner surface of the small intestine.


The objective of our study was to appraise 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. *domesticus*) 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 large-scale piggeries with different management.

Pathological studies were carried out in piglets whose contained oocysts of *Isospora suis*. Feaces were examined by flotation employing Schäfer's solution (500 g sugar, 320 ml water, 5 ml phenol).

Bacteriological examinations were performed in order to exclude specific pathogens. In two breeds, administered enroflaxacin, Rotavirus and enteropathogenic strains of *E. coli* was confirmed by electronmicroscopy. In one breed, a concurrent infection by adenoviruses was ascertained elsewhere (Vitovec et al. 1988). In another breed, *Isospora suis* was the only pathogen producing disease.

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 ileocecal. More specimens were collected at 15, 20, and then at each point distant successively 30 cm from ostium ileocecal 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 histological examinations were collected from the liver, kidneys, spleen, lung, brain, pancreas, regional mesenterial lymph nodes, and eventually the colon. Histology revealed no pathological alterations or moderate changes usually of nonspecific character, such as serosal thickening, adipose tissue, fibrous thickening or dysplasia. 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 on a microscope slide, and stained the smears to evaluate incidences of endogenous stages of *Isospora suis* by Stevenson and Andrews (1982) and Lindsay et al. (1988). Samples from the gut or other organs were examined histologically to fix at 10% neutral formalin. The material was prepared by routine histological methods. Sections were stained by haematoxylin. The eosin stain employed to identify protozoa in the gut, alcin blue and PAS reaction to determine mucopolysaccharidices. Semi-thin sections were prepared by means of ultramicrotome LKB 3 and LKB 4 A. We stained them by toluidine blue and also polychromatically according to Warńczewski and Shau 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 22 piglets, one piglet aged 8 days, 1 piglet 9 days, 7 piglets 10 days, 6 piglets 11 days, 2 piglets 11 days, one piglet 13 days, and 4 piglets 14 days. Animals older than 14 days excreted no or very few oocytes, and lesions were moderate while signs of postinflammatory reparative predominated.

The macroscopically manifest consequences of natural isosporosis are inflammatory or cellular reactions which are often associated with the formation of cysts, abscesses and granulomas. These changes are often associated with the formation of cysts, abscesses and granulomas. These changes are usually permanent and may be accompanied with the development of oedematous membranes that become adherent to the mucosa of the ileum.

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 metastatic pavement epithelium (Plate I, Figs. 1, 2). Sometimes the apex of necrotic villi was covered by pseudomembranes composed of a fibrin network and a number of degenerating epithelial cells, regressive cells elements of inflammatory infiltration and numerous endogenous stages of *I. suis* (Plate II, Figs. 1, 2, Plate III, Figs. 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 hyperemic, oedematous 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 masses which were negative when stained with alcin blue, Graham-Black PAS. Exposed to trichromic staining, some parts of the necrotic mass acquired blue colouring while other a red one indicating so that they partly consisted of hyalized structureless protein material.

With this category of piglets (aged 8 to 11 days), the stroma of both atrophic and ulcerated villi was covered by crypts whose cytoplasm was markedly bright, vacuolized, and voluminous. The stroma in these areas was thin and edematous, cells incidentally contained substances which reacted positively to alcin 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 the merozoites mostly identified spherical or ovoidal meronts of different stages of maturity. Less often encountered were ovoidal or extended microgamonts bearing on their peripheries bases for microgametocytes. Frequent were both immature and mature oocysts. In azur-eosine, the cytoplasm 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 ileocecal, 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 ileocecal we identified exclusively meronts while sectors situated more cranially 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 tangential layer of epithelium. They were normally only equally numerous in 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 Peyers' patches (Plate III, Fig. 3).

The endogenous stages of *Isospora suis* were located in vacuoles within the cytoplasm 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 semimembraneous bulge of the cell wall.

Piglets aged 13 to 14 days presented from the pathological point of view an incongruous group. In one case of a piglet aged 14 days, we observed pseudomembranous...
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 intergrows 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 occurring from day 7 to day 10. Our trials placed alterations due to isosporosporidiosis 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. (1980) 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.

Parasite populations affected 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: fibro-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 20 to 140 cm cranially from ostium ileocecal. 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 duodenal portion of jejunum. Their number also 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 cytoplasm. 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 intesti-
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Fig. 1. Epithelial metaplasia (arrow) and erosions in apical parts of jejunal villi affected with endo-
genous 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 ecoc-
cosms caused by I. suis. (HE, 300×). Fig. 2. Pseudomembranes and necroses of apical parts of jeunal
villi in natural ecocosis 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 parabasalophorous vacuole imbedded below the nuclei of enterocytes located in jejunal vili. (HE, 600×).

Fig. 4. Endogenous stages of *I. suis* implanted in submucosal lymphatic tissue of Peyer's patches in ileum. (Azur-cosine, 700×).

Fig. 1. Gamonts of *I. suis* within villous jejunal epithelium. Semi-thin section. (Warmke and Sieu-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 semi-spherical bulge of the cell wall. Semi-thin section. (Warmke and Sieu-Ling Janet Lee, 1,400×).