Coccidiosis in Swine: Dose and Age Response to *Isospora suis*

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ABSTRACT

Coccidiosis is a disease of the young piglet due to infection with *Isospora suis* and is characterized by diarrhea which is nonresponsive to antibacterial therapy. There is variable morbidity and mortality. Piglets develop a more severe clinical illness and enteritis when infected with *I. suis* at one to three days of age than when infected at two weeks of age. Microscopic lesions range from villous atrophy and mild erosion to severe fibrinonecrotic enteritis.

RÉSUMÉ

La coccidiose est une maladie des porcelets, imputable à une infection par *Isospora suis*; elle se caractérise par une diarrhée qui ne répond pas à une antibiothérapie. La morbidité et la mortalité affichent des variations. Les porcelets développent une coccidiose clinique plus grave et une entérite, lorsqu'on les infecte dès l'âge d'un à trois jours plutôt qu'à deux semaines. Les lésions microscopiques varient d'une atrophie et d'une légère érosion des villosités intestinales à une entérite fibrino-nécrotique.

INTRODUCTION

Coccidiosis has become a commonly diagnosed enteric disease in swine. *Isospora suis* has been associated with the clinical disease seen in piglets less than two weeks of age (15). The disease is characterized by scours, variable mortality and morbidity and is nonresponsive to most antibacterial therapy (12). In Georgia, peak incidence occurs in late summer and fall (13). The effects of *I. suis* in piglets dosed at one day of age with sporulated oocysts have been described (14). This report compares the clinical and pathological response to *I. suis* in older piglets to the lesions previously reported in one day old piglets.

MATERIALS AND METHODS

An inoculum of *I. suis* was recovered from a piglet with clinical disease and subsequently was placed in parasite free donor piglets for collection of large numbers of oocysts. Collected oocysts were separated from fecal debris through a series of sieves, harvested via centrifugation at 400 g in Sheather's sugar solution, sporulated in 2.5% potassium dichromate at room temperature and stored at 4°C.

Piglets used in this study were farrowed at the Veterinary Diagnostic and Investigational Laboratory at Tifton, Georgia. Sows and piglets were confinement raised on commercial plastic covered Tenderfoot floors (Oriole, Inc., Blooming Prairie, Minnesota). All sows received Amprolium® (Merck and Company, Rahway, New Jersey) for seven to ten days prior to farrowing at the rate of 10 mg/kg body wt/day. There were no coccida in the feces of the sows at the time of farrowing.

Piglets were given access to baby pig creep feed, weaned at two weeks of age, and were kept in individual stainless steel cages with collection pans. These early weaned pigs received milk replacer and baby pig creep feed. Piglets in groups I received either 200,000 or 400,000 sporulated oocysts of *I. suis* at one day of age, group II received either 200,000 or 400,000 at three days of age, group III received 400,000 at two weeks of age, group IV received 2,000,000 at four weeks of age or 1,000,000 at six weeks of age (Table I).

The feces were examined daily for oocysts by flotation in Sheather's sugar solution and oocysts per gram (OPG) of feces. Piglets were killed when comatose or at five to 14 days postdosing. The small intestine (sectioned at 30 cm intervals), large intestine, mesenteric lymph nodes, spleen, liver, kidney,

TABLE I. Coccidiosis in Swine: Sporulated Oocysts of *Isospora suis* Given to Piglets at Various Ages

Group	Number Piglets	Age Piglets	Oocyst Dose
I	9	1 day	200,000
	17	1 day	400,000
	6	1 day	Control
II	24	3 day	200,000
	3	3 day	400,000
	6	3 day	Control
III	13	2 weeks	400,000
	11	2 weeks	Control
IV	3	4 weeks	2,000,000
	2	6 weeks	1,000,000

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lung and heart were collected in 10% (pH 7.3) phosphate buffered formalin. Tissues fixed in formalin were routinely processed and 4 to 5 μm sections were prepared and stained with hematoxylin and eosin. Tissue from the duodenum, jejunum and ileum were also fixed in 2.5% glutaraldehyde at pH 7.3 with secondary fixation in 1.0% phosphate buffered osmium tetraoxide. Fixed blocks of intestine were dehydrated in increasing concentrations of ethanol and critically-point dried in a Denton DCP-I critical point drying apparatus (Denton Vacuum Inc., Cherry Hill, New Jersey) and mounted on stubs. They were coated with 100 nm gold-palladium in a Technics Hummer I sputter coater (Technics Co., Alexandria, Virginia). The specimens were examined with a ETEC Omniscan scanning electron microscope (ETEC Corporation, Hayward, California).

RESULTS

Piglets in group I which received 200,000 oocysts at one day of age developed severe diarrhea three to four days after dosing and shed oocysts (16,700 mean OPG) for four to five days. Piglets which received 400,000 oocysts at one day of age also developed severe diarrhea, became dehydrated and comatose at three to five days after dosing with high (70%) mortality. At necropsy a diphtheritic membrane was seen in the jejunum and ileum in two of the nine piglets receiving 200,000 oocysts as compared to nine of 17 piglets receiving 400,000 oocysts at one day of age. Severe necrosis of intestinal villi with adhered fibrinonecrotic cellular debris was seen microscopically in piglets which had gross lesions. Microscopic lesions in piglets without a diphtheritic membrane were characterized by marked villous blunting, fusion and mild to marked erosion. Similar changes are demonstrated with scanning electron microscopy in Fig. 1. Normal intestinal villi are illustrated in Fig. 2.

Fig. 1. Intestinal villi from mid-jejunum of a one day old piglet given 200,000 sporulated oocysts of *Isospora suis* and killed five days postdosing. Note the short, broad villi with fusion and marked erosion of the tips. X168.

Twenty-four piglets (three litters) in group II received 200,000 oocysts at three days of age. These piglets had been used as untreated controls in an earlier anticoccidial efficacy study. The piglets developed yellow diarrhea at three to four days postdosing which generally lasted two to four days and alternated with a grey pasty or semiformed stool. Two piglets comatose at seven and ten days postdosing had oocyst counts of 758,400 OPG of feces on day 7 postdosing and 135,240 mean OPG for five days, respectively. A diphtheritic membrane was seen in the jejunum and ileum. Nine other piglets necropsied at five to eight days postdosing had no gross lesions but on microscopic examination had mild to moderate erosion of villous epithelium and villous atrophy, particularly within the mid and distal jejunum. Oocyst counts varied from 18,900 to 412,800 OPG of feces collected from these nine piglets at necropsy. The 13 remaining piglets which were not necropsied had a mean oocyst count of 30,014 OPG of feces over an average patent period of five days. The three piglets in group II which received 400,000 sporulated oocysts at three days of age had severe diarrhea at three days postdosing and became dehydrated and gaunt. No death occurred prior to six days and the piglets were killed six, seven and eight days postdosing. A diphtheritic membrane was seen at necropsy and marked villous necrosis was seen microscopically in the most severely affected piglet killed at six days postdosing. This piglet had 13,900 OPG of feces collected at necropsy. Moderate to marked villous atrophy and epithelial erosion was seen microscopically in the jejunum and ileum in the other two piglets. In these latter piglets the average daily oocyst count was 9000 OPG of feces for three days.

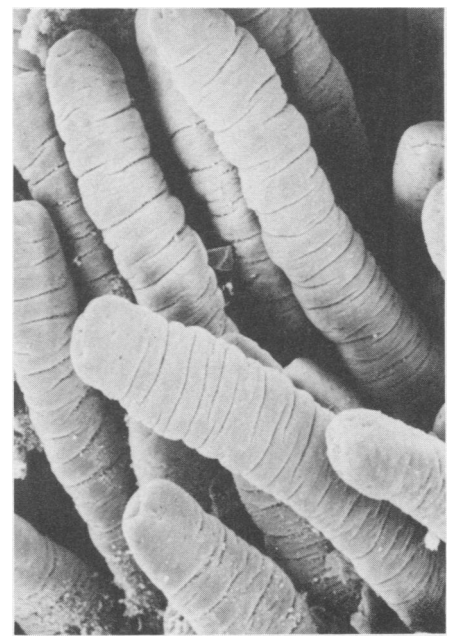

Fig. 2. Intestinal villi from mid-jejunum of a one week old control piglet. Note the elongate fingerlike smooth appearance with an intact extrusion zone at the villous tip. X82.

Thirteen piglets in group III dosed at two weeks of age with 400,000 oocysts developed mild to moderate diarrhea at three to four days postdosing. The diarrhea, which often alternated with pasty or formed stools, lasted four to six days. No gross lesions were seen at necropsy. Microscopic changes in three piglets killed at four, five and six days postdosing were mild epithelial erosion with mild to

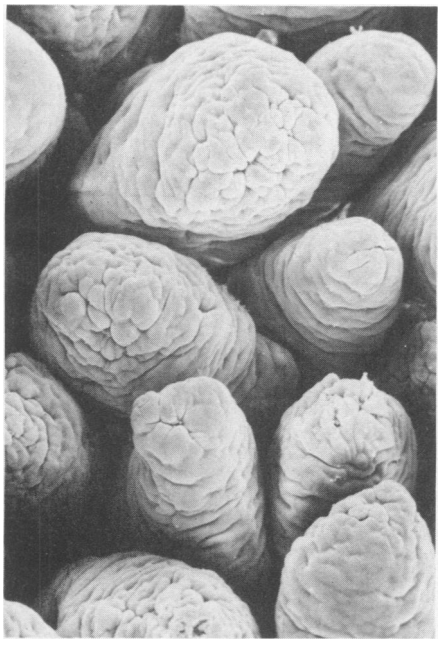

Fig. 3. Intestinal villi from mid-jejunum of a two week old piglet given 400,000 sporulated oocysts of *Isospora suis* and killed five days postdosing. Note the minimal villous blunting and intact villous tip. X116.

Fig. 4. Intestinal villi from the same piglet and region of jejunum as described in Fig. 3. There is greater blunting and fusion but minimal erosion of the tips. X116.

moderate villous atrophy in the mid-jejunum. There were increased numbers of lymphocytes and plasma cells in the lamina propria of the jejunum. No oocysts were seen in the feces of two piglets killed at four days postdosing. The piglet killed at five days postdosing had 17,250 OPG of feces and the piglet killed six days postinoculation had 50,267 OPG of feces. Scanning electron microscopy confirmed the mild villous blunting (Figs. 3 and 4) contrasted to villi of a control piglet (Fig. 5) and to the severe blunting and erosion seen in piglets dosed at one to three days of age. The seven remaining piglets shed oocysts for three to five days with a mean daily oocyst count of 160,044 OPG of feces. The piglets had alternating mild diarrhea and pasty to formed feces.

In group IV, mild clinical scours occurred in three piglets four weeks of age which were dosed with 2,000,000 oocysts and in two piglets six weeks of age given 1,000,000 oocysts. No gross or significant microscopic lesions were seen at necropsy. The mean OPG for a four day patent period in the three piglets dosed with 2,000,000 was 49,863. The pigs dosed with 1,000,000 shed oocysts for eight days but no OPG was determined.

DISCUSSION

Coccidiosis as a clinical entity occurs in piglets less than two weeks of age (12,13). Although *I. suis* is often recovered from clinically affected piglets, it is seldom found in older swine (16). However, oocysts of many *Eimeria* sp. are routinely found in older swine in the absence of clinical disease (16).

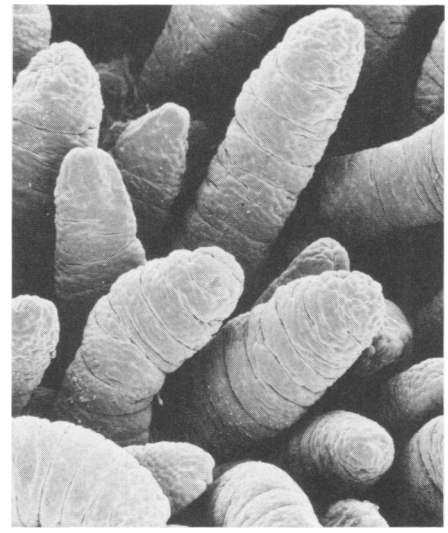

Fig. 5. Intestinal villi from mid-jejunum of a three week old control piglet. Note the normal fingerlike elongate shape with an intact tip. X80.

The response of piglets to *I. suis* in this study depended on the oocyst dose and age of the piglet. The clinical and pathological effects of *I. suis* were most marked in piglets one to three days of age as compared to piglets two weeks of age and older. A diphtheritic membrane within the jejunum and ileum was often present in the young piglets receiving 200,000 to 400,000 oocysts while no gross lesions were observed in the older (two to six weeks of age) piglets dosed with 400,000 to two million sporulated oocysts. Microscopically, a severe fibrinonecrotic enteritis was seen in the younger piglets, whereas only mild villous atrophy and epithelial erosion were noted in the older piglets.

The effects on body weight, oocyst discharge, and abnormality of fecal discharge in calves given *Eimeria bovis* are directly proportional to the dose (5). A "crowding effect" is reported with large doses of oocysts where insufficient numbers of epithelial cells are available for multiplication, resulting in fewer numbers of oocysts passed. The "crowding effect" is reportedly more noticeable in immunogenic species of coccidia (8,11). In our experience, with challenge dosing, it appears that *I. suis* in piglets is most immunogenic. The decrease in oocyst production with large doses of oocysts may also be related to interferon or an interferon-like substance produced in response to the coccidia (8,11). The high dosages of oocysts given to older piglets in this study produced minimal clinical disease, with oocyst counts less than or comparable to lesser doses given to younger piglets. Older animals reportedly provide a more favorable environment for parasite multiplication (11) than younger animals, with greater oocyst passage as demonstrated in chickens

with *E. maxima* (7) or *E. tenella* (10).

In some studies, clinical disease, mortality, decreased weight gain and definitive pathological lesions of coccidiosis were more easily produced in younger hosts, as in turkeys using *E. adenoeides* and *E. meleagridis* (1,2,8) or *E. acervulina* in chickens (6). Other studies have shown that very young animals are more resistant to infection than older ones (3,7,10). For example, calves three weeks of age are reported to be more resistant to *E. alabamensis* than older calves (3). Rose suggests that the comparative inefficiency of excysting mechanisms in the very young (10,11) along with other host factors such as the deficiency of paraminobenzoic acid associated with a milk diet are responsible for adversely affecting the growth of the parasite (11).

Isospora suis has been reported to cause clinical disease in older pigs rather than in young piglets (9). Yet, in our experience and that of other diagnosticians (4), the clinical disease occurs in the young nursing piglet.

Additional studies are needed to determine the residual effect of *I. suis* on intestinal morphology and subsequent absorptive function in piglets and on the immune response to repeated infection.

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