BIOLOGY AND PATHOGENICITY OF EIMERIA SPINOSA HENRY, 1931 IN EXPERIMENTALLY INFECTED PIGS

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Abstract—Koudela B. and Vitovec J. 1992. Biology and pathogenicity of Eimeria spinosa Henry, 1931 in experimentally infected pigs. International Journal for Parasitology 22: 651-656. A single-species isolate of E. spinosa from a diarrheic weaned pig was used to determine the endogenous development and pathogenicity of this swine coccidium. Seven out of 14 inoculated pigs developed endogenous stages or passed oocysts of E. spinosa in their feces. Immunosuppressive treatment with cyclophosphamide had no effect on the susceptibility to infection with E. spinosa in young pigs. The endogenous stages developed within the apical cytoplasm of the enterocytes lining the distal part of the villi in the posterior jejunum. The asexual development comprised three generations of meronts, which were seen at 5, 7 and 9 days post-infection (DPI). Meronts of the first generation measured 6-8 μm and produced 10-14 merozoites 4-6 μm in length. The second generation of meronts measured 6-8 μm and contained 10-20 merozoites 4-6 μm in length. Third generation mature meronts (8-10 μm) on DPI 9 contained 12-20 merozoites measuring 5-7 μm, which were more crescent-shaped and less blunt than the merozoites at 5 and 7 DPI. Merogony continued after formation of the gametes and the first fully developed macrogametes (10-14 μm), microgametes (9-12 μm), and oocysts were also seen at 9 DPI. The prepatent period was 8 or 9 days, but the patent period was not determined. In the present study E. spinosa infection did not produce overt clinical signs. Pathological changes consisted of an inflammatory infiltration in the lamina propria of the posterior jejunum, Peyer’s patches activation and sporadic erosions scattered at the villous tips. No villous atrophy in association with a large number of endogenous stages was observed.

INDEX KEY WORDS: Coccidiosis; pig; pathogenicity; endogenous development; Eimeria spinosa.

INTRODUCTION

Little is known concerning the biology and the pathogenicity of the swine coccidium Eimeria spinosa because little experimental work has been carried out on this parasite. Wiesenhiutter (1962) inoculated a single 8-week-old pig with 6000 sporulated oocysts on each of 2 successive days, and, more recently, Ernst (1987) reported attempts to infect 19 4-week-old pigs with different doses of oocysts of E. spinosa. The aim of our study was to investigate the endogenous development, pathogenicity and the effect of immunosuppressive treatment on the susceptibility to infection with this coccidium in young pigs.

MATERIAL AND METHODS

Eimeria spinosa oocysts. A single-species isolate of E. spinosa was obtained from a naturally infected pig (approx. 60 kg body weight) with diarrhea. This pig was estimated to have passed about 3 million oocysts per g of feces over 8 days.

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Some fecal material from this pig was strained through cheese cloth and allowed to settle. The sediment was mixed with 2.5% aqueous potassium dichromate solution and kept in Petri dishes at 20-23°C. After sporulation the oocysts were stored in potassium dichromate solution at 4°C until used.

Pigs and experimental inoculation. Sixteen pigs were used in this study; they were housed in farrowing crates and given commercial feed that did not contain a coccidiostat and water ad libitum. The first group of pigs (crossbred Large White (×) Landrace) consisted of four 5-week-old animals from the same litter. These pigs were inoculated via a stomach tube with a suspension of an estimated 5,000 sporulated oocysts of E. spinosa in PBS. Two pigs were killed and necropsied at 8 and two at 9 days post-infection (DPI). To check the possible role of immunosuppression on the coccidial infection, one of each pair was given cyclophosphamide (Sigma) at a daily dose of 5 mg kg⁻¹ administered intramuscularly 3 days before and during the course of infection up to time of necropsy. Pigs of the second group (12 pigs from the same litter) were Durocs. Three days before and during the course of infection five pigs were given cyclophosphamide (CY) at the same dose as the pigs of the first group. Ten 33-day-old pigs (five treated with CY and five
untreated) were inoculated with 500,000 oocysts of *E. spinosa*. They were killed and necropsied, in pairs, at 3, 5, 7, 9 and 11 DPI. Two pigs served as a control, and were not killed.

**Examination of pigs.** Pigs were observed daily for clinical signs of coccidiosis. Feces of each pig were examined daily post-inoculation for oocysts using flotation in Sheather's sugar solution. At necropsy the intestinal tract was opened and macroscopically examined for gross pathology. The samples for histology were taken first from the ileum within 5 cm of the ostium ileocecale (OIC). The other intestinal samples were removed at intervals of 50 cm cranially from the OIC in such a way that the last specimen originated from the duodenum. In the large intestine specimens were collected from the apex of the cecum, the colon near the ansa centralis, and the rectum. Additional samples for histology were taken from the liver, kidneys, spleen, lungs, pancreas and regional mesenteric lymph nodes. The material was fixed in 10% neutral buffered formalin and processed routinely. Histological sections were stained with hematoxylin and eosin or azure and eosin. The inner surface of the intestine was examined by scanning electron microscopy (SEM) using 4% phosphate-buffered (pH 7.2) paraformaldehyde-fixed material. Small strips of intestinal epithelium were rinsed several times in distilled water and dehydrated in an ascending ethanol series. Material for SEM was critically point-dried under CO₂, coated with gold-palladium and viewed in a TESLA BS 300 SEM. As a part of the necropsy we took mucosal scrapings from different portions of the intestine. Mucosal smears were fixed in methanol and stained with Giemsa to evaluate the incidence and morphology of endogenous stages of *E. spinosa*. The samples of ileum, mesenteric lymph nodes, spleen, liver, kidneys and lungs were bacteriologically cultured in order to exclude specific pathogens.
Fig. 5. Second generation meronts in the epithelium of the posterior jejunum. 7 DPI. H&E. Scale bar, 8 μm.

Fig. 6. Oocysts (Ooc), sexual (Se) and asexual (As) developmental stages located at the tips of the villus. 9 DPI. H&E. Scale bar, 5 μm.

Fig. 7. Scattered monocellular erosions of the villous tips in the posterior jejunum. 7 DPI. SEM. Scale bar, 100 μm.
RESULTS

Only seven of the 14 experimental pigs developed infections (two in the first group and five in the second). Of these, one (killed at 9 DPI) in the first group and two (killed at 5 and 7 DPI) in the second group were given cyclophosphamide. Neither of the control pigs developed patent infections.

The oocysts of *E. spinosa* were found to be ovoid, occasionally ellipsoidal with a brown, rough, spined wall composed of two layers, about 2 μm thick and without a micrype (Figs. 1 and 2). The sporulated oocysts were without an oocyst residuum but with a polar granule, and measured 20.3 (x) 14.2 (15-25 (x) 12-26) μm. The sporocysts were elongate ovoid with a prominent Stieda body and a residuum, and they measured 11.1 (x) 6.1 (10-13 (x) 5-7) μm.

Oocysts of *E. spinosa* in feces collected from the experimentally infected pigs were suspended in 2.5% potassium dichromate solution and were kept at a 20–23°C temperature. Sporulation began on the 12th day, when the first oocysts with the protoplasm divided into four round masses were seen, and was completed within 17 days. About 70% of the sporulated oocysts were normal in appearance. The remainder had not sporulated and appeared morphologically abnormal, with hyaline globules within a single concentrated protoplasmic mass, and the oocyst wall was collapsed and thinner than normal.

The endogenous development of *E. spinosa* was found to take place in the posterior jejunum in the portion 50–200 cm distant from the OIC. Parasites were located in the apical cytoplasm above the host nucleus within the enterocytes lining the distal part of the villi. No parasites were found in the goblet cells, in the base of the villi, or in the crypts. No parasites were found in the sections of the cecum, colon or rectum or in the other organs examined.

Endogenous stages of *E. spinosa* were first seen in mucosal smears and sections from the posterior jejunum 5 DPI. At this time post-infection, developing multinucleate spherical meronts and mature meronts were found (Figs. 3 and 4). In smears, the immature meronts were seen to contain eight to 12 nuclei arranged on the periphery and measured 4–8 μm. The mature meronts measured 6–8 μm and contained 10–14 crescent-shaped merozoites. These measured 5.2 (x) 1.2 (4–6 (x) 1.0–1.5) μm in smears. At 7 DPI, immature spherical meronts were found. They contained only two to four nuclei and measured 4–6 μm. The mature meronts (6–8 μm) were prolific at this time post-infection (Fig. 5). As before, each was in a parasitophorous vacuole and contained 10–20 crescent-shaped merozoites. A residual body was not observed in these mature meronts. The uninucleate merozoites were of the same morphology as the merozoites at 5 DPI. No sexual stages were seen in smears or histological sections until 8 DPI when a few immature macrogamonts (3–5 μm) were found.

At 9 DPI, merogony continued after the formation of gametes (Fig. 6). The distribution of stages was 32% mature meronts, 42% macrogamonts, 9% mature macrogametes, 6% developing microgamonts and 11% oocysts. The third generation mature meronts (8–10 μm) contained 12–20 merozoites that filled the parasitophorous vacuole. These merozoites measured 6.3 (x) 1.2 (5–7 (x) 1.0–1.5) μm and were more crescent-shaped and less blunt than the merozoites at 5 and 7 DPI. A residual body was not found in these meronts. Developing macrogamonts were elongate ovoid and measured 4–14 (x) 4–9 μm. Mature macrogametes (10–14 μm) in sections stained with azur and eosin were characterized by their granular eosinophilic cytoplasm. Mature microgamonts (9 12 μm) contained peripherally arranged microgametes and a centrally located residual body. Oocysts in sections contained an eosinophilic sporont and were distinguished from mature macrogametes by their oocyst wall (Fig. 6). No endogenous stages were seen in pigs necropsied at 11 DPI.

Oocysts of *E. spinosa* were first seen in the feces of one pig without CY treatment at 8 DPI. This pig that was necropsied at 11 DPI passed only a small number of *E. spinosa* oocysts, and many of them had a collapsed wall and hyaline bodies in the sporont. Oocysts of *E. spinosa* were first seen at 9 DPI in the feces of the other two pigs to become infected. In the present study the patent period of *E. spinosa* was not determined.

None of the inoculated pigs developed clinical signs of coccidiosis or died and pathogenic bacteria were not isolated from any of the pigs. No macroscopic pathological changes were found at necropsy in pigs killed at 3 and 5 DPI. At necropsy of the infected pigs killed at 7, 8 and 9 DPI the wall of the posterior jejunum was slightly thickened. The mucosa was hyperemic and the regional mesenteric lymph nodes were edematously enlarged.

Using histopathological and SEM examination of the inner intestinal surface, no pathological changes were found in immunosuppressed pigs nor in pigs without CY treatment on the first 3 days post-infection. In the immunosuppressed pig, killed at 5 DPI, endogenous stages of *E. spinosa* were observed in the epithelium of the posterior jejunum. In the lamina propria of this portion of the intestine a discrete inflammatory infiltrate was observed. Changes in the inner surface did not occur at this time post-infection. Endogenous stages of *E. spinosa* were observed in the one untreated and one immunosuppressed pigs examined at 7 DPI. The lamina propria of the posterior
jejunum with the endogenous stages of *E. spinosa* was edematous and hyperemic, permeated with infiltrate and the Peyer's patches were enlarged and activated. SEM examination showed scattered monocellular erosions of the villous tips in the posterior jejunum (Fig. 7). At 8 DPI, asexual stages of *E. spinosa* were seen in the one untreated pig. Pathological changes observed in this pig were identical to those found in pigs at 7 DPI. However, no villous atrophy in association with the large number of endogenous stages was seen in the posterior jejunum at 9 DPI in the untreated but infected pig. The distribution of parasites along the villi and their location within the enterocytes were identical to those observed in the pig examined in previous days post-infection. The lamina propria contained an inflammatory infiltrate and the Peyer’s patches were enlarged and activated. Neither of the pigs examined at 11 DPI had endogenous stages of *E. spinosa* in tissue sections. Fecal flotation from the pig without CY treatment examined at 11 DPI contained a small number of *E. spinosa* oocysts. Pathological changes observed in this pig were identical to those observed at 9 DPI.

Concurrent *Giardia* infection was found in seven pigs of the second group. No pathological changes were detected in any of them. The flagellates were found in tissue sections, smears and by SEM examination in four of five animals treated with CY and in three of five without treatment (Koudela, Nohýnková, Vitovec, Pakandl & Kulda, 1991).

**DISCUSSION**

The swine coccidium *E. spinosa* was first described by Henry (1931) from the cecal contents of two pigs. She said that there were numerous oocysts present. Andrews & Spindler (1952) stated that naturally infected pigs passed between 1 and 4 million and up to 7 million oocysts of *E. spinosa* per g feces. In this study, a naturally infected pig passed, during 8 days of observation, about 3 million oocysts of *E. spinosa* per g feces.

The characteristics of the oocysts of *E. spinosa* as given in published descriptions were compared with those of the oocysts found in our investigation. The oocysts described above are similar to those described by Henry (1931), Vetterling (1965) and recently by Löwenstein & Kutzer (1989). The spinous wall is typical and diagnostic for this species of swine coccidium.

The oocysts of *E. spinosa* collected from the naturally and experimentally infected pigs completed sporulation within 17 days at 20–23°C. Henry (1931) stated that the sporulation time of *E. spinosa* was 12 days and Wiesenmüller (1962) stated that it could be 15–22 days, but neither author gave the temperature of sporulation. Boch, Pezenburg & Rosenfeld (1961) reported that the sporulation time of *E. spinosa* at 23–25°C was 15–17 days. Löwenstein & Kutzer (1989) reported that the sporulation time of *E. spinosa* at 25°C was 9–10 days. These differences between reports of the sporulation time of *E. spinosa* may be due to several factors such as laboratory techniques, different incubation temperatures and lack of adequate oxygenation. In addition, in the case of the *E. spinosa*, the sporulation time is difficult to determine satisfactorily because of the brown colour and the opacity of the oocyst wall caused by the spines.

In the course of incubation at room temperature for 20 days, about 70% of *E. spinosa* oocysts sporulated and the rest of them degenerated. These degenerated oocysts possessed hyaline globules and their wall was abnormally thin. These observations are similar to those of two other authors (Wiesenmüller, E., unpublished inaugural dissertation, Freie Universität, Berlin, 1962; Ernst, 1987). Wiesenmüller believed that the morphologically abnormal oocysts of *E. spinosa* were excreted early from enterocytes and they were too immature to sporulate.

Little is known concerning the endogenous development of *E. spinosa*. Only two reports of experimental work that has been done on this parasite have been found. Wiesenmüller (1962) described the results of an infection in a single 8-week-old pig. Ernst (1987) inoculated 19-4-week-old pigs with different doses of *E. spinosa* oocysts. Only four pigs passed oocysts of *E. spinosa* in their feces, and he concluded that *E. spinosa* did not readily infect pigs. In the work described here we have examined the possibility that relative resistance to infection with *E. spinosa* in pigs is immunologically mediated. Seven of the 14 inoculated pigs were immunosuppressed with a low dose of CY. Immunity to coccidia is expressed through the activity of CD4+ T lymphocytes (Wakelin & Rose, 1990) and low-dose CY treatment induces a depletion of T lymphocytes (Rach, 1975). Only seven of the 14 pigs that were inoculated developed endogenous stages or passed oocysts in their feces: two of seven immunosuppressed and four of seven of CY-untreated pigs. Clearly, the pig is a relatively unsuitable host for *E. spinosa* and the relative resistance or susceptibility to *E. spinosa* in pigs is not immunologically mediated.

Although eight species of *Eimeria* and a single *Isospora* species have been described and are widespread in the swine population (Vetterling, 1965), relatively little is known about endogenous development of swine coccidia. The complete endogenous development of *E. debliecki* has been described by Vetterling (1966). Rommel & Ipczynski (1967) studied the endogenous development and pathogenicity of *E. scabra* and Lindsay, Stuart, Wheat & Ernst (1980) investigated the endogenous development of *I. suis*.

The single pig that Wiesenmüller (1962) infected with *E. spinosa* had asexual and sexual stages in the
jejunal and ileal and large numbers in the intestinal content. He found the endogenous stages in the jejunal from 100 cm posterior to the stomach to the jejunum (E. scabra and I. suis) by their smaller size.

In the endogenous development of E. spinosa we found three asexual generations of meronts. The mature meronts at 5 DPI are considered the first generation of meronts. Those at 7 DPI, the time of greatest concentration of asexual stages of E. spinosa in the intestine, are the second generation and those at 9 DPI are the third. Thus, merogony continued after the first formation of gametes, which were the most frequent in our material at 9 days after inoculation. These findings could explain the long-term oocyst output in pigs naturally infected with E. spinosa (Henry, 1931; Andrews & Spindler, 1952; our observation of the naturally infected pig).

In the present study, E. spinosa did not produce overt signs of coccidiosis in any of the inoculated pigs, and the conclusion of Ernst (1987) that E. spinosa does not cause diarrhea in weaned pigs was confirmed. The moderate pathological changes of the intestinal mucosa did not influence the clinical state of experimentally infected pigs. Continuing damage of the mucosal barrier did not occur and this is probably the reason for the subclinical course of infection.

The swine coccidium E. spinosa was first described from the cecum of the pig which showed lesions similar to those found in enteritis in swine (Henry, 1931). Wiesenhüter (1962) inoculated a single pig with oocysts of E. spinosa, which died 11 days later. The death of this heavily infected pig is misleading, because Wiesenhüter (1962, dissertation cited above) revealed that it was sickly before inoculation. These data show that clinical signs and pathological changes associated sometimes with E. spinosa infection in young pigs may be a result of the interaction of coccidiosis and other diseases.

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REFERENCES


