Biology of *Isospora* spp. from Humans, Nonhuman Primates, and Domestic Animals

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INTRODUCTION

*Isospora* species are protozoan parasites that are in the phylum Apicomplexa. They are members of the group of organisms referred to as coccidia. The term “coccidia” was once used to refer primarily to members of the genera *Eimeria* and *Isospora* but is now used to include *Cryptosporidium* species, *Toxoplasma gondii*, and other members of the suborder Eimeriina. Coccidia have complex life cycles. Members of the genus *Isospora* can complete their entire life cycle in a single host. Several have evolved the ability to use a paratenic host (transport host) in their developmental cycle.

Coccidia are identified to the species level based on the structure of their sporulated oocyst stage. The size, shape, color, texture, and type of internal contents are important features used in identifying coccidial oocysts. The oocyst stage is an environmentally resistant stage which is excreted in the
Isospora species can cause serious disease in humans and nursing pigs. Clinical disease is seldom seen in nonhuman primates, dogs, or cats. Isospora species do not produce disease in horses, domestic ruminants, rabbits, or domestic poultry, and reports of isosporan oocysts in the feces of these hosts probably represent pseudoparasites that originated in feed contaminated with wild-bird feces.

**TAXONOMIC PROBLEMS**

The sporulated oocysts of *Isospora* species resemble the sporulated oocysts of the related genera, *Toxoplasma*, *Hammondia*, *Besnoitia*, *Frenkelia*, and *Sarcocystis*. This resemblance led to much confusion during the period from the late 1800s to the mid-1970s, when the life cycle of these parasites was not known. We will consider the two most notable examples in which these problems cause confusion.

*Isospora hominis*

Human isosporiasis is caused by *Isospora belli*, which is a true member of the genus *Isospora* (187). Many early reports of human coccidiosis refer to infection with a parasite described as *Isospora hominis*. *I. hominis* is actually a species of *Sarcocystis*, and the name is a synonym for *Sarcocystis hominis* or *S. suihominis*, species acquired by ingesting rare or raw infected beef or pork, respectively. There is no structural means of differentiating these two species of *Sarcocystis* in human fecal samples or in intestinal biopsy specimens. Intestinal sarcocysts in humans can be a serious disease (18), unlike in other animals, which normally show no clinical signs. In many early reports, it is impossible to determine whether the authors are describing *I. belli* or *Sarcocystis* species. An example of this confusion can be found in the pioneering work on coccidiosis, *Coccidia and Coccidiosis of Domesticated, Game and Laboratory Animals and of Man*, by E. R. Becker, published in 1934 (7). Becker includes line drawings that demonstrate sporation of *I. belli* but refers to the parasite as *I. hominis*. The only way one can be certain if early authors are describing *I. belli* or *I. hominis* (*Sarcocystis*) is to examine the drawings or photomicrographs if present. If none are included, a definitive identification may not be possible.

*Isospora bigemina*

In the early and mid-1900s, it was thought that dogs and cats shared the same species of coccidia (7, 8). The name *Coccidium bigemina* had been given by Stiles in 1891 to a parasite developing in the lamina propria of a dog (39). The organism was placed in the genus *Isospora* in 1906. Based on the location of the sporocysts, the parasite observed by Stiles was obviously a species of *Sarcocystis*. Wenyon believed that there were two "races" of *I. bigemina* that could be differentiated based on size and called them the large and small races of *I. bigemina* (188). The large race developed in the lamina propria and was excreted as sporulated oocysts or sporocysts (i.e., *Sarcocystis* species), whereas the small race developed in the epithelial cells of the small intestine and was excreted as unsporulated oocysts. It is clear now that the small race of *I. bigemina* in dogs is actually *Hammondia heydorni*, an obligatorily heteroxenous parasite (81). It is impossible to determine what the small race of *I. bigemina* in cats actually was because its oocysts are structurally indistinguishable from those of *Toxoplasma gondii*, *Hammondia hammondi*, and *Besnoitia* species. Reports of the large race of *I. bigemina* in cats, other animals, and humans also actually refer to *Sarcocystis* species.
LIFE CYCLE

Coccidial life cycles are complex, with both exogenous and endogenous cycles present. Paratenic (transport) hosts may also be employed.

Ultrastructure

Transmission electron microscopy has been widely used to examine the developmental stages of coccidial parasites. The entire life cycle of *I. suis* in pigs has been described by using TEM, and it was similar to that described for *Eimeria* species (123). Notable differences are present in the structure of the sporozoite stages of *Isospora* and *Eimeria* species. The sporozoites of mammalian *Isospora* species contain one or two inclusions, termed crystallloid bodies, that are composed of particles similar in appearance to beta-glycogen particles, whereas the sporozoites of *Eimeria* species contain one or two inclusions, termed refractile bodies, that appear to be proteinaceous. These inclusions are generally lost in the process of conversion from sporozoite to merozoite stage (59, 122) in vivo but may persist in parasites cultivated in vitro (107).

Sporogony

Sporogony is the production of infective sporozoites within sporocysts inside the oocyst. Sporogony usually occurs outside the host and is the exogenous phase of the coccidial life cycle. Sporogony is dependent on moisture, temperature, and adequate oxygen. Several controlled studies have been conducted on the sporogony of *Isospora* oocysts from dogs (94, 117), cats (160), and pigs (108). These studies indicate that temperatures greater than 40°C or less than 20°C inhibit sporulation of the oocysts. Rapid sporulation (<16 h) of oocysts occurs at 30 or 37°C. Structural events that occur during sporogony are similar for all species. Oocysts are excreted in the feces, and they usually have a contracted sporont. A few oocysts will be excreted in the sporoblast stage (two-celled stage). As the nucleus of the sporont divides, a clear nuclear streak is formed, nuclear division occurs, and the sporont divides to form two uninucleate sporoblasts. Nuclear division occurs again, and the nuclei are visible as clear areas at the poles of the sporoblast. Nuclear pyramids may be seen at the poles of the sporoblasts. The sporoblasts become elongate and form the sporocyst stage. Nuclear division occurs again, and the outline of developing sporozoites soon becomes visible. When the sporozoites are fully visible, the oocyst is considered to be sporulated. A small percentage (<2%) of oocysts are *Caryospora*-like and contain one sporozoite which encloses eight sporozoites (96, 108, 121, 193) (Fig. 1b). Heat treatment of unsporulated *I. rivolta* oocysts at 50°C for 5 min increased the numbers of *Caryospora*-like oocysts that were produced after sporulation (121). These *Caryospora*-like oocysts of *I. rivolta* were infectious for mice (paratenic hosts) and cats. Oocysts collected from cats inoculated with *Caryospora*-like oocysts were *Isospora*-like after sporulation, indicating that heat treatment did not induce a stable mutation. The biological significance of these *Caryospora*-like oocysts is unknown.

Excystation

Excystation is the process by which sporozoites are released from the sporocysts/oocysts. The process is basically the same for all mammalian *Isospora* species studied to date (46, 47, 109, 128, 165, 166) and is similar to what occurs in *Sarcocystis* spp. (15) and *T. gondii* (22). Studies have been conducted with intact oocysts or with sporozoites that have been mechanically freed from the oocyst wall. Pretreatment of oocysts with sodium hypochlorite solution (109, 165) or cystine hydrochloride (128) under CO₂ enhances excystation of intact oocysts. Exposure of oocysts or sporocysts to sodium taurocholate solution (0.75%, wt/vol), bile (5%, vol/vol), or sodium taurocholate (0.75%, wt/vol) plus trypsin (0.25%, wt/vol) will cause activation of sporozoites. If bile is used, the host animal from which the bile is obtained is of little importance (128). Sporozoites become motile within the sporocysts and tumble or glide around one another. This movement is not continuous but is interrupted by periods of inactivity. Eventually, the sporocyst wall opens along four plate-like junctions (148, 165, 166) and the sporozoites will exit through the opening that is formed. Sporozoites exit oocysts through indentations or fractures that form in one or both ends of the oocyst wall (128, 165).

Endogenous Development

The endogenous life cycle of mammalian *Isospora* species is somewhat different from that of typical *Eimeria* species (Fig. 2). Sporozoites enter cells in the intestine but usually do not form rounded uninucleate trophozoites. Some sporozoites and/or merozoites leave the intestine and form dormant cyst stages (hypnozoites) in extraintestinal tissues (31, 40, 149). Intestinal sporozoites may retain their elongate sporozoite shape, become binucleate, and divide by endodyogeny to form two daughter merozoites. These daughter merozoites divide by endodyogeny an indefinite number of times (27, 59, 107, 122, 123). For this reason, the number of sequential asexual merozoogonous cycles cannot be determined, and developmental stages are referred to as structural types instead of generations. Eventually, multinucleate meronts are formed. These meronts are elongate and retain their merozoite shape. Several meronts may occur in the same host cell, and, with time, sexual stages are formed. Microgamonts are multinucleate and produce biflagellated microgametes (60). Macrogamonts lack the highly cosinophilic wall-forming bodies found in most *Eimeria* species, and the oocyst wall is usually inconspicuous. Microgamonts and macrogamonts may coexist in the same host cell.

The endogenous life cycles in animals that ingest oocysts and in those that ingest paratenic hosts are similar (35, 38). The prepatent period may be shortened in infections that are initiated by consumption of paratenic hosts (35, 38, 43, 65).

Extraintestinal Stages

Extraintestinal stages occur in the tissues of the definitive host in canine and feline *Isospora* species (31, 40) and *I. belli* of humans (130, 149) (Fig. 3 and 4). Instead of undergoing the normal developmental cycle in the intestinal tract, some sporozoites (merozoites?) leave that site and invade extraintestinal sites in the host. Mesenteric lymph nodes are most often involved, but other tissues such as the liver, spleen, and tracheobronchial and mediastinal lymph nodes can be infected. Parasites are usually found as single organisms resembling sporozoites, but some division may occur at these extraintestinal sites, and up to 15 parasites have been observed in an infected cell (40). The infected host cells probably are macrophages.

Mice, rats, hamsters, dogs, cats, cattle, sheep, and camels have been shown to be paratenic hosts for several *Isospora* species (31, 40, 42, 55, 65, 78, 82, 154, 192). Sporozoites excyst from oocysts and invade extraintestinal tissues. Mesenteric lymph nodes are most often infected; other tissues such as the spleen, liver, and skeletal muscles are sometimes parasitized. Parasites are most often found as single organisms; parasite division at these sites has not been confirmed (65). For this
FIG. 2. Developmental stages of *I. rivolta* in cats and mice. (A, B, G to J, M, and N) Smears fixed in methanol and stained with Giemsa. (C to F) Sections stained with iron hematoxylin (C, D, F) and by the PAS reaction (E). (K and L) Smears not fixed or stained. (A and C) Division of meronts by endodyogeny (arrow). (B) An immature meront with four nuclei. (D) Two multinucleated meronts (arrows) in the same parasitophorous vacuole. (E) PAS-positive granules (arrow) in merozoites. (F) Meronts with different-sized merozoites (arrows). (G) An immature microgamont with many nuclei (arrow). (H) Several mature microgametes (arrow). (I) Macrogamont with a large nucleus (arrow) and prominent nucleolus. (J) An unsporulated oocyst. (K) Unsporulated oocyst containing a contracted sporont. (L) Sporulated oocyst containing two sporocysts with sporozoites (arrows). (M) Extraintestinal zoites in the mesenteric lymph node of a cat. One zote is in a host cell (arrow), and one has ruptured out of its host cell (arrowhead). (N) Extraintestinal tissue cyst containing a single zote (arrow) in the mesenteric lymph node of a mouse. Magnifications, ×2,300. Reprinted with permission of the publisher from reference 38.
reason, it is more accurate to refer to the host as a paratenic rather than an intermediate host.

Transmission electron microscopy reveals that the sporozoites are inside a parasitophorous vacuole (PV) (14, 42, 129) (Fig. 3). The appearance of the contents of the PV changes during the course of infection. At 1 day postinoculation (p.i.) sporozoites are surrounded by a PV membrane that has a wavy appearance, and the PV contains numerous vesicles. By 7 days p.i., there is an electron-dense granular layer immediately beneath the PV membrane. Filaments or tubules may also be present in this layer. It is this granular layer that appears as a thick wall by light microscopy. Membrane-bound, electron-dense granules, apparently of host cell origin, are present at the margins of the PV membrane. The sporozoite lies in the center of the cyst. Sporozoites increase in size during the course of infection and accumulate polysaccharide granules in their cytoplasm. It is because of the presence of these polysaccharide (amylopectin?) granules that the sporozoites stain positively in the periodic acid-Schiff (PAS) reaction. The crystalloid bodies of sporozoites remain intact during the course of the infection.

Disease does not occur in paratenic hosts (38). Parasites remain viable for at least 23 months in extraintestinal tissues of mice (38). When the definitive host ingests a paratenic host, the subsequent prepregnant period may be shorter than when infections are initiated by oocysts. The number of oocysts produced by the definitive host and the patent period are similar to those in oocyst-induced infections (43). The tissues of paratenic hosts are not infectious for other paratenic hosts (38).

An interesting interaction occurs in mice experimentally infected with I. felis and then challenged with Babesia microti. Mice infected with I. felis 28 days before infection with B. microti do not develop B. microti antibodies but are completely resistant to infection with B. microti (176). Partial resistance to B. microti can be achieved by transfer of spleen cells from mice infected with I. felis. Treatment of I. felis-infected mice with a monoclonal antibody to L3T4+ cells increases their susceptibility to B. microti infection (176). These results suggest that cell-mediated immunity is involved in the observed nonspecific resistance.

DEVELOPMENT IN VITRO

Several mammalian Isospora species have been grown in cell cultures (54, 56, 57, 58, 102, 107). Primary cell cultures from the host animal generally support the most numerous and most chronologically advanced parasite stages. Sporozoites are obtained from excysted oocysts and used as an inoculum. Sporozoites penetrate host cells and undergo several divisions by direct penetration of the host cell membrane. Filaments or tubules may also be present in the PV layer, which is the granular layer that appears as a thick wall by light microscopy. Membrane-bound, electron-dense granules, apparently of host cell origin, are present at the margins of the PV membrane. The sporozoite lies in the center of the cyst. Sporozoites increase in size during the course of infection and accumulate polysaccharide granules in their cytoplasm. It is because of the presence of these polysaccharide (amylopectin?) granules that the sporozoites stain positively in the periodic acid-Schiff (PAS) reaction. The crystalloid bodies of sporozoites remain intact during the course of the infection.

The diagnosis of coccidiosis in animals is based on clinical signs (diarrhea), history, evaluation for potential copathogens, and demonstration of coccidial oocysts of a pathogenic species in the animals' feces. Knowing the actual numbers of oocysts present in the feces is of little help in determining if clinical disease is present.

Demonstration of parasite stages in tissue samples collected at necropsy in animal infections or in intestinal biopsy specimens or samples collected at autopsy in human infections is also suitable for obtaining a diagnosis. Special stains are of little value in identifying coccidial stages. Familiarity with the appearance of the stages is far more useful in locating them in histological samples (Fig. 2).

ISOSPORA INFECTIONS OF HUMANS

I. natalensis has been reported in humans (48), but little is known about this parasite. It was found in the feces of a 21-year-old patient suffering from amebic dysentery and other protozoal and helminth infections. Oocysts of I. natalensis were observed on four consecutive days (after the patient had been treated for amebic dysentery), and the I. natalensis infection was self-limiting. Infection with this parasite has apparently not been observed since 1953, when it was described. Its oocysts resemble those of the I. ohioensis complex seen in dogs, I. rivolta of cats, and I. suis of pigs, but they are slightly larger (Table 1).

I. chilensis described from humans in South America is not a valid name; it is a species of Sarcocystis. As mentioned above, I. hominis is also no longer considered a valid name because it too is a species of Sarcocystis.

Three cases of infection with a coccidian species believed to be an isosporan were reported from humans in Papua New Guinea (4). The oocysts were excrated unsporulated, were spherical, and measured 8.5 μm in diameter. Sporulation was slow, taking about 2 weeks, and the final proportion of oocysts that sporulated was only about 10%. The sporocysts of this coccidium were illustrated in drawings with no Stieda body, but there appears to be a Stieda body in the photomicrographs that accompany the description. The parasite is probably a species of Cyclospora, a recently recognized coccidial pathogen of humans that has two sporocysts with Stieda and sub-Stieda bodies that enclose two sporozoites (144).

ISOSPORA BELLI INFECTIONS

I. bellii infections are essentially cosmopolitan in distribution but are more common in tropical and subtropical regions, especially Haiti, Mexico, Brazil, El Salvador, tropical Africa, the Middle East, and Southeast Asia (53, 88, 164). Pigs, dogs,
mice, rats, rabbits, guinea pigs, and rhesus monkeys are not suitable definitive hosts (61, 87); however, in one study, patent infections were reported in two of three gibbons (193). This lack of susceptibility has led some researchers to discount animals as reservoirs (90). However, it is not known if these or other animals may serve as paratenic hosts for *I. belli*. The role of paratenic hosts in the transmission of *I. belli* needs to be investigated to establish whether modes of transmission other than by contaminated food or water exist. The existence of paratenic hosts may help explain infections occurring in areas where sanitation is adequate.

**Life Cycle of *I. belli***

Oocysts are passed in feces unsporulated or partially sporulated (sporoblast stage). They can sporulate in less than 24 h (133). Oocysts are elongate and ellipsoidal with slightly tapered ends, or one end may be tapered and the other end blunt (Fig. 1; Table 1). The patent period is not known. It may be as little as 15 days in some patients (127). Chronic infections develop in some patients, and oocysts are excreted for several months to years. In one case, an apparently immunocompetent individual had symptoms that were present for 26 years and had *I. belli* infection documented on several occasions over a 10-year period.

All life cycle stages typical of *Isospora* species have been observed by light and transmission electron microscopy (16, 149, 179). The number of asexual types present has not been determined. If the life cycle is similar to that of other carnivor/omnivore *Isospora* species, the first asexual division would be by endodyogeny. Division by endodyogeny probably occurs repeatedly. Endogenous stages are located in enteroctyes lining the villi of the small intestine and rarely in those in the large intestine (16, 149, 179). Endogenous stages are seldom found in other locations such as enteroctyes lining the crypts or in cells in the lamina propria. Extraintestinal infections have been observed in AIDS patients (see below) and probably also occur in immunocompetent patients.

**Intestinal Infections in AIDS Patients**

Diarrhea produced by *I. belli* in AIDS patients is often very fluid and secretory-like and leads to dehydration requiring hospitalization. Fever and weight loss are also common findings. Other opportunistic pathogens are also common in these patients. Intestinal lesions induced by *I. belli* and the responses to chemotherapy are usually similar to those in immunocompetent patients.

In an extensive 8-year surveillance program of AIDS patients in Los Angeles County (164), *I. belli* was found in 127 (1%) of 16,351 patients. The prevalence of infection was highest among foreign-born patients, especially patients from El Salvador (7.4%) or Mexico (5.4%) or of other Hispanic ethnicity (2.9%). Patients between the ages of 14 and 24 were more likely to have *I. belli* infection than were older patients. Patients with a history of *Pneumocystis carinii* pneumonia were less likely to have *I. belli* infection. The authors concluded that isosporiasis among AIDS patients in Los Angeles may be related to travel exposure and/or recent immigration from Latin American countries. Additionally, the use of trimethoprim (TMP)-sulfamethoxazole (SMX) for the treatment or prevention of *P. carinii* pneumonia may effectively prevent the acquisition of primary *I. belli* infection or the recrudescence of existing *I. belli* infection. It was recommended that physicians have an increased index of suspicion for *I. belli* in AIDS patients with diarrhea who have immigrated from or traveled to Latin America, are Hispanics born in the United States, are young adults, or have not received prophylaxis with TMP-SMX for *P. carinii*. Additionally, it was suggested that AIDS patients traveling to Latin America and other developing countries be advised of the potential for food-borne and waterborne acquisition of *I. belli* infection and consider taking TMP-SMX chemoprophylaxis.

*I. belli* infection was observed in 20 (15%) of 131 AIDS patients with opportunistic infections in Port-au-Prince, Haiti (28). Stool samples collected from 170 siblings, friends, and sexual partners were negative. No demographic or laboratory characteristics distinguished patients with AIDS and *I. belli* from patients with AIDS and other opportunistic infections. In another study, three of three patients with *I. belli* infection were from Haiti and lived in the United States at the time of the study (190).

Nine (19%) of 46 patients from Zaire with chronic diarrhea and suspected of having AIDS had *I. belli* (80). Eight of the nine *I. belli*-positive patients were later confirmed to have AIDS. *I. belli* was found in 13 (9.9%) of 81 AIDS patients examined at a reference center in Sao Paulo, Brazil (158). Stool samples from 81 immunocompetent individuals were negative for *I. belli*. Three (5%) of 60 AIDS patients examined in Catalina, Spain, were positive for *I. belli* oocysts (155).

A pregnant AIDS patient with *I. belli* diarrhea diagnosed at 5.5 months of pregnancy delivered a live human immunodeficiency virus-positive infant (147). Her sexual partner was also...

**TABLE 1. Measurements of oocysts of *Isospora* species from mammals**

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Dimensions (μm):</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Oocysts&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>I. belli</em></td>
<td>Humans</td>
<td>23–36 by 12–17</td>
</tr>
<tr>
<td><em>I. natalensis</em></td>
<td>Humans</td>
<td>24–30 by 21–25</td>
</tr>
<tr>
<td><em>I. arcopithecus</em></td>
<td>NH primates&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21–30 by 21–25</td>
</tr>
<tr>
<td><em>I. callimico</em></td>
<td>NH primates</td>
<td>13–21 by 12–17</td>
</tr>
<tr>
<td><em>I. scorzi</em></td>
<td>NH primates</td>
<td>23 by 20</td>
</tr>
<tr>
<td><em>I. canis</em></td>
<td>Dogs</td>
<td>34–40 by 28–32</td>
</tr>
<tr>
<td><em>I. ohiensis</em></td>
<td>Dogs</td>
<td>19–27 by 18–23</td>
</tr>
<tr>
<td><em>I. brunowi</em></td>
<td>Dogs</td>
<td>17–22 by 16–19</td>
</tr>
<tr>
<td><em>I. rivolta</em></td>
<td>Cats</td>
<td>18–28 by 16–23</td>
</tr>
<tr>
<td><em>I. felis</em></td>
<td>Cats</td>
<td>38–51 by 27–39</td>
</tr>
<tr>
<td><em>I. suis</em></td>
<td>Pigs</td>
<td>17–25 by 16–21</td>
</tr>
</tbody>
</table>

<sup>a</sup>Measurements represent the range unless none was reported.

<sup>b</sup>NH primates, nonhuman primates.
positive for *I. belli*. Treatment with TMP-SMX never eliminated the *I. belli* infection.

**Extraintestinal Infections in AIDS Patients**

Two reports of disseminated extraintestinal isosporiasis in patients with AIDS have been published (130, 149). The first patient was a 38-year-old white male homosexual who was examined at the National Institutes of Health, Bethesda, Md. (149). He initially presented to a local hospital with a history of progressive dyspnea and fever; he also complained of dysphagia, nausea, vomiting, and brown watery diarrhea (eight or nine episodes daily). He had lost 20 lb (9.17 kg) in 2 months (15% of his body weight). *P. carinii* pneumonia and oropharyngeal candidiasis were noted, and he was treated with TMP-SMX and pentamidine. His condition improved, and he was discharged 24 days after admission. He subsequently was readmitted complaining of nausea, vomiting, and diarrhea. He was diagnosed as having *Giardia lamblia* infection and was treated with metronidazole. Five months after his initial hospitalization, he was diagnosed as having *I. belli* and *Entamoeba histolytica* infection. He was treated with TMP-SMX, metronidazole, and dideoquin. Three months later he presented with dyspnea, fever, diarrhea, and generalized wasting. Cytomegalovirus pneumonia was demonstrated at this time. Repeated stool examinations were negative. He died 2 weeks later. At autopsy, the body demonstrated severe cachexia, focally consolidated lungs, multiple small intestinal foci of multifocal erythema and hemorrhage, ulcerated cecal lesions up to 5 mm across, and enlarged mesenteric, periaortic, and mediastinal lymph nodes. Microscopically, disseminated cytomegalovirus infection involving the lungs, intestines, adrenal glands, mesenteric lymph nodes, and, to a lesser extent, liver and pancreas was observed. *Mycobacterium kansasi* was cultured from the liver and spleen, although no granulomas were observed in tissue sections.

Microscopic findings associated with *I. belli* infection were observed in the lymph nodes and walls of both the small and large intestines. Marked lymphocytic depletion was observed in the lymph nodes, and foci of granuloma-like histiocytic proliferation were seen in the mesenteric, periaortic, and mediastinal lymph nodes. Intracellular zoites were observed in the cytoplasm of histiocytes. The parasites were surrounded by a thick eosinophilic cyst wall in hematoxylin-and-eosin-stained sections. The cyst wall was PAS positive. Most of the infected cells contained only one zoite; however, some contained two or three. Examination of the intestinal tissues demonstrated intraepithelial asexual and sexual stages of *I. belli* and occasionally merozoites that appeared to be in cells in the lamina propria. Numerous *I. belli* oocysts were observed in scrapings obtained from the intestine.

The second case was observed in a 30-year-old black woman who was a native of Burkina Faso but had lived in France for 2 years (130). She initially presented with fever, diarrhea, and weight loss. She was found to have esophageal candidiasis and *I. belli* infection. The *I. belli* infection was treated with TMP-SMX (200 mg/day), and the diarrhea resolved within a week. She was placed on maintenance therapy of 100 mg of TMP-SMX daily but suffered eight episodes of recurrent infection diagnosed by stool examination or duodenal biopsy over the next 3 years. Examination of the biopsy specimens demonstrated severe villous atrophy and crypts, gamonts, and oocysts of *I. belli* within enterocytes. Gamonts and merozoite-like stages were observed in the lamina propria. No other pathogens were observed in the biopsy specimens. An autopsy conducted after her death revealed cachexia. The abdominal cavity contained 0.5 liter of serous ascitic fluid. The liver, spleen, and mesenteric lymph nodes were enlarged. The small intestine and colonic mucosa were pale and atrophic, but no ulcerations or perforations were present. No gross lesions were observed in the omentum or other tissues. Examination of samples collected at autopsy revealed stages of *I. belli* in the intestine, mesenteric and mediastinal lymph nodes, liver, and spleen (Fig. 4). The extraintestinal stages were always observed as single organisms that did not stain with acid-fast stains. The tissue cyst wall was PAS negative, but the enclosed zoite contained PAS-positive granules. The tissue cyst wall did not stain by the Gomori-Grocott method. Massive infection was observed in the lymph nodes in association with plasmacytosis and some eosinophils but no granulomatous reaction. Parasites were usually grouped in clusters in the paracortical areas or the lumen of the sinus. Few parasites were observed in Kupffer cells or within macrophages located in portal areas. No involvement of the biliary system was noted. A moderate steatosis and cholestasis was also observed. The spleen had *I. belli* tissue cysts in the red and white pulp; the cysts were associated with congestion and atrophy of the white pulp.

Notable differences in the light microscopic findings in these two patients are the presence of more than one zoite within a tissue cyst observed in the first patient and the lack of PAS reactivity of the tissue cyst wall in the second. Additionally, no granulomatous reaction was observed in the lymph nodes in the second patient. It was believed that the concurrent cytomegalovirus infection helped lead to dissemination of the parasite in the first patient. However, cytomegalovirus or other intestinal pathogens were not documented in the second patient.

Transmission electron microscopy was used to examine portions of lymph nodes in both patients, and the findings were similar. The zoites were in a PV within the cytoplasm of histiocytes. Organelles typical of coccidial sporozoites/merozoites with a crystallloid body and polysaccharide granules were present. An electron-dense granular layer was seen immediately beneath the PV membrane. This layer probably composed the tissue cyst wall observed by light microscopy. The ultrastructural features of these tissue cysts observed in the lymph nodes of humans are similar to the tissue cysts observed in mice inoculated with *I. felis* and *I. ohioensis*.

A recent study (23) examined the submicroscopic appearance of *I. belli* infection in a 30-year-old white female intravenous drug user from Italy who had AIDS. Her symptoms were...
watery, nonbloody diarrhea and fever. She was treated with TMP-SMX, and her diarrhea stopped in 2 days. No other clinical data were presented. Ultrastructural examination of small intestinal biopsy specimens taken at the duodenoejunal junction demonstrated trophozoites, merozoites, meronts, and macrogamonts in epithelial cells. Occasionally, merozoites were observed in the intestinal lumen, in the lamina propria, and within lymphatic channels. The demonstration of merozoites in lymphatic channels documents a means of their dissemination to lymph nodes and to other tissues. The authors considered that their findings of extracellular merozoites might indicate that *I. belli* is not strictly an intracellular parasite. This consideration is erroneous, because it is well documented that motile stages of *Isospora* can leave host cells and invade new host cells (110). This movement is a normal part of the life cycle, and these fortuitous observations of extracellular stages are not indicative of extended extracellular survival by these forms of the parasite. It is interesting that the photomicrograph of a merozoite in a lymphatic channel (Fig. 6 in reference 23) appears to be a tissue cyst. The merozoite is surrounded by electron-dense material identical to that seen in tissue cysts in lymph nodes.

Asexual and sexual stages and oocysts of *I. belli* have been observed in the bile duct epithelium of an AIDS patient with acalculous cholecystitis (8a). No lymph nodes were examined in this patient, and the relationship between bile duct infections and disseminated infections with tissue cysts is presently not known.

**Infections in Other Immunocompromised Hosts**

Clinical disease in *I. belli* infections is usually more severe in immunocompromised patients than in immunocompetent patients. *I. belli* has been observed in patients with concurrent Hodgkin's disease (16), non-Hodgkin's lymphoproliferative disease (72), human T-cell leukemia virus type 1-associated adult T-cell leukemia (68), and acute lymphoblastic leukemia (189). These patients respond to specific anti-*I. belli* treatment (see below).

It was suggested in one report that treatment with prednisolone (60 mg/day for 13 days) led to transient immunosuppression and severe *I. belli* infection in one patient (134). The patient recovered without specific treatment.

**Infections in Immunocompetent Hosts**

*I. belli* causes serious and sometimes fatal disease in immunocompetent humans. Symptoms of *I. belli* infection include diarrhea, steatorrhea, headache, fever, malaise, abdominal pain, vomiting, dehydration, and weight loss (16, 75, 85, 98, 179). Blood is not usually present in the feces. Eosinophilia is observed in some patients. The disease is often chronic, with parasites present in the feces or biopsy specimens for several months to years. Recurrences are common.

Experimental infections demonstrate that fever begins 8 days after ingestion of oocysts and lasts for about 8 days (120). Nonbloody diarrhea begins 7 to 9 days after ingestion of oocysts. The prepatent period is 10 to 11 days, and oocysts are excreted for 32 to 38 days. No oocysts were excreted when one volunteer attempted to reinfect himself 33 days after ingestion of oocysts, indicating that immunity had developed.

Disease is more severe in infants (98) and young children (178) than in adults. A 6-month-old male infant in California had *I. belli* infection that terminated fatally after 30 weeks of continuous total parental nutrition (98). The disease was characterized by severe diarrhea (1 to 3 liters daily) due to cholera-like hypersecretion of intraluminal fluid. Little clinical response to surgical, dietary, or antibiotic treatments was observed. An 18-month-old female in Thailand was admitted to hospital with severe dehydration, inappetence, and weakness (178). She had four or five diarrheic bowel movements daily. She responded to treatment with electrolytes and TMP-SMX, and her diarrhea ceased within 5 days.

**Microscopic Lesions Due to *I. belli***

The main microscopic changes are villous atrophy and crypt hyperplasia (16, 149, 179). Eosinophils may be present in the lamina propria in large numbers approaching those seen in eosinophilic enteritis. Plasma cells, lymphocytes, and polymorphonuclear leukocytes (PMNs) are present in increased numbers. The lymphatics may be dilated.

**Diagnosis**

The Sheather sugar flotation method is an excellent method for detecting oocysts of *I. belli* (26, 115). The unsporulated oocysts of *I. belli* are readily visible unstained by light microscopy. Oocysts are in a slightly higher plane of focus than other parasite cysts or ova (49). Flotation methods are superior to direct fecal smears for detecting oocysts (53). Sedimentation concentration methods are also more sensitive than direct smears. Charcot-Leyden crystals may (70, 88, 162) or may not (163) be present in stool samples that contain *I. belli* oocysts.

Stained fecal smears made from concentrated samples may aid in the detection of *I. belli* oocysts (17, 92, 115, 137, 145). The modified acid-fast stain produces pink-staining oocysts that contain bright red sporonts or sporoblasts (137). Oocysts stained by the auramine-rhodamine procedure fluoresce bright yellow (115). When the Giemsa stain is used, both the oocysts and sporoblasts stain pale blue. The heated safranin-methylene blue technique produces oocysts that are orange-red (17). The trichrome stain is of little use (92).

Duodenal aspirates (100, 179), the duodenal string test (190), and small intestinal biopsies (179) are also useful in suspected cases in which oocysts are not found in stool samples. *I. belli* oocysts are observed in duodenal aspirates and in mucus collected in the string test. Developmental stages of *I. belli* can be identified in enterocytes in small intestinal biopsy specimens. Some biopsy samples may be negative for developmental stages but contain characteristic lesions. Likewise, oocysts may be present in stool samples from some biopsy-negative patients (63). Routine histological staining methods are satisfactory for demonstrating parasite stages. Many of the parasites will be in vacuoles, making them readily identifiable. *I. belli* can cause disease with relatively few stages of the parasite present and can be missed on small intestinal biopsy.

**Treatment**

Many agents have been used to treat *I. belli* infections. Combinations of protozoal dihydrofolate reductase/thymidylate synthase inhibitors (TMP or pyrimethamine) with sulfonamides (SMX, sulfadiazine, or sulfadoxine) have generally proven effective. Treatment with TMP-SMX has been used most often (23, 28, 62, 88, 92, 147, 178, 189). One study examined the TMP-SMX treatment of a group of 32 Haitian AIDS patients. The patients ranged in age from 24 to 55 years old. They had a history of chronic intermittent diarrhea with a mean duration of 7.9 months (range, 2 to 26 months). The diarrhea was liquid, and 2 to 10 stools were excreted a day. The patients also had a history of diffuse, crampy abdominal pain, nausea, and intermittent fever. Of the 32 patients, 28 required...
oral or intravenous rehydration before or during the first 3 days of the study. The patients were treated with oral TMP (160 mg)-SMX (800 mg) four times a day for 10 days. Diarrhea and abdominal pain stopped 1 to 6 days (mean, 2.5 days) after treatment. All stool samples examined after the end of treatment were negative. At the end of the study, the prophylaxis of I. belli infection was examined in these patients. Ten patients received placebo orally three times a week, 10 received TMP (160 mg)-SMX (800 mg) orally three times a week, and 12 received pyrimethamine (25 mg)-sulfadoxine (500 mg) orally once a week. Of the 10 patients given placebo, 5 developed recurrent I. belli infection in 1 to 3.5 months and were retreated with TMP-SMX for 10 days with favorable outcomes. None of the patients given pyrimethamine-sulfadoxine had treatment recurrenday47 after treatment.

**ISOSPORA INFECTIONS OF NONHUMAN PRIMATES**

Little is known about the coccidial infections of nonhuman primates. Most of the Isospora species recorded are known only by their oocyst structure (Table 1).

I. callimico was isolated from the feces of a Goeldi's marmoset (Callimico goeldii) at a laboratory animal facility in Baltimore, Md. (Table 1) (84). The oocysts were excreted for 7 days and sporulated in 2 days.

I. endocallimico was isolated from the feces of five Goeldi's marmosets from the Tulane University Delta Regional Primate Research Center in Louisiana (Table 1) (46). Two of the animals were born at the center, and three were exported from Peru. No transmission or life cycle studies have been conducted with these species.

I. scorzai was isolated from the feces of a Uakari monkey (Cacajao rubicundus) that was housed in the London Zoo, and the parasite was transmitted to another monkey, Cebus nigrivittatus (2). The life cycle of I. scorzai is not known. Experimentally inoculated kittens did not excrete oocysts.

I. cebi was isolated from the feces of a Cebus albifrons from the Alto Magdalena region of Colombia (119). The sporocysts of this species have Stieda bodies, indicating that it is a pseudoparasite of avian origin. A similar Isospora species was isolated from the feces of a Bonnet monkey (Macaca radiata) at the Delhi Zoo in India but was not named (9).

Isospora paponis was isolated from Chacma baboons (Papio ursinus) (125). Oocysts sporulated endogenously in the small intestines, indicating that this is a Sarcocystis species. Additionally, sporulated oocysts of this species have been seen in the skeletal muscles of Chacma baboons (126). Chimpanzees (Pan troglodytes) can also serve as definitive hosts for Sarcocystis species, and reports of Isospora sporocysts in their feces actually describe Sarcocystis sporocysts.

**I. arctopitheci Infections**

I. arctopitheci has been studied more than the other coccidia of nonhuman primates (76–78, 140). Hendricks conducted cross-transmission studies with this parasite and claimed to have successfully transmitted it to members of six genera of New World nonhuman primates, four families of carnivores, and one marsupial species (77). This is an unusually small and diverse definitive host range, and further experimental studies are needed to confirm or deny these initial findings.

The endogenous life cycle of I. arctopitheci occurs in the small intestine (140). Developmental stages are located in enterocytes on the distal two-thirds of the villi, and parasite densities are greatest in the jejunum. Asexual multiplication was found to be exclusively by endodyogeny, and ecosinophilic bodies were present in gamonts (140). The prepatent period was about 7 days, but the patent period was not reported. Extraintestinal stages were not seen in the definitive host.

Experimental studies indicate that I. arctopitheci can be pathogenic (140). Of 13 titi marmosets (Saguinus geoffroyi), 4 died after being inoculated with $1 \times 10^5$ to $2 \times 10^9$ oocysts. No clinical signs were seen in marmosets that died 3 and 5 days p.i. Bloody diarrhea was seen in two marmosets that died 7 days p.i. All nine other marmosets remained normal. The micro-
Diagnostic and Treatment

Diagnosis is made by finding the characteristic oocysts (Table 1) in fecal samples. Fecal flotation with Sheather’s sugar solution is recommended as a reliable and sensitive technique. Sedimentation or other concentration techniques are also adequate.

Most Isospora infections in nonhuman primates are subclinical. We are unaware of any reports on the treatment of Isospora infections in nonhuman primates. Agents used in humans or veterinary products may be of some value.

**ISOSPORA INFECTIONS OF DOGS AND CATS**

**Infections of Dogs**

Several species of Isospora infect dogs (Table 1). Cats are not the definitive hosts for *Isospora* species found in dogs (32). Young dogs are more likely to be infected, and surveys indicate that 3 to 38% of dogs are positive for coccidial oocysts (91). Stray dogs are more likely to be infected than are dogs with owners because stray dogs must hunt for food and therefore have more exposure to infected paratenic hosts.

It is unclear if coccidiosis is a serious problem in dogs (103, 146). Diarrhea associated with the presence of coccidial oocysts in young dogs occurs, but the clinical significance is not established because of the possibility of concurrent bacterial or viral infections. Published reports of naturally occurring canine coccidiosis are few (24, 44, 141), and further studies on natural cases are needed before firm conclusions can be made. Experimental infections have not usually been associated with disease.

*I. canis* infections. *I. canis* has the largest oocysts of the canine *Isospora* species and is the only species that can be diagnosed by microscopic examination of oocysts (Table 1). *I. canis* develops in cells in the lamina propria of the posterior small intestine (93). Three asexual types are present, and the first asexual division is probably by endodyogeny. The prepatent period is 9 days. The length of the patent period has not been determined.

Disease was not produced in 25 6-week-old or 6 8-week-old pups inoculated with 1 × 10⁸ to 1.5 × 10⁸ *I. canis* oocysts (93). Solid immunity follows primary infection, and no oocysts are discharged after challenge (46). It has been suggested that the stress of weaning and shipping may enhance *I. canis* infections (93). This suggestion needs further investigation because these outbreaks of coccidially associated diarrhea may be related to a decrease in immunity and reactivation of latent extraintestinal stages with subsequent intestinal infection and clinical signs of disease.

The *I. ohioensis* complex. Three *Isospora* species having smaller oocysts than *I. canis* can be found in dogs: *I. ohioensis*, *I. burrowsi* (Table 1), and *I. neorivolta*. Because they cannot be separated based on oocyst structure and because *I. ohioensis* was the first named, these oocysts are often referred to as *I. ohioensis*-like (44) or members of the *I. ohioensis* complex.

*I. ohioensis* develops in enterocytes in the small intestine, cecum, and colon of dogs (35). Two asexual types are recognized, and division by endodyogeny is observed. The prepatent period is 5 days, and the length of the patent period is not known. The parasite can cause disease in experimentally infected 7-day-old pups but not weaned pups or young dogs (36). Diarrhea was the major clinical sign seen in the 7-day-old pups. Microscopic changes were villous atrophy, necrosis of apical enterocytes, and cryptitis. Dogs developed an immunity that lasted for about 2 months.

*I. burrowsi* develops in enterocytes and cells in the lamina propria in the posterior small intestine (180). Two asexual types are present. Division by endodyogeny has not been recorded but probably occurs. The prepatent period is 6 days, and oocysts are excreted for 9 to 15 days.

*I. neorivolta* develops in cells in the lamina propria in the posterior small intestine (41). Four asexual types are recognized, and division by endodyogeny is observed. The prepatent period is 6 days, and oocysts are excreted for 13 to 23 days.

Little is known about the pathogenesis of *I. burrowsi* or *I. neorivolta* infection in dogs. Neither caused disease in experimental infections of dogs (41, 116, 180).

Because the significance of diarrhea caused by coccidia in dogs is unclear, the treatment of the condition is also unclear. Suspected clinical cases can be treated with a variety of drugs used alone or in combination (see below).

**Infections of Cats**

*I. rivolta* and *I. felis* infect cats. Dogs do not serve as definitive hosts for these species (159). Both feline *Isospora* species have extraintestinal stages in the feline definitive host and in a variety of paratenic hosts. From 3 to 36% of cats examined excrete oocysts (91). Stray cats are more likely to excrete oocysts. Coccidiosis in cats is not thought to be a common problem (191) and is usually seen only in naturally infected kittens in which other disease-causing agents may be present. The drugs used to treat dogs are used to treat kittens.

*I. rivolta* infections. *I. rivolta* develops in enterocytes in the small intestine (38). Three structural types of asexual stages are present. The first asexual division is by endodyogeny. The prepatent period is 4 to 7 days, and oocysts are excreted for more than 14 days.

Experimentally, *I. rivolta* can cause disease in newborn kittens (38). Diarrhea occurs 3 to 4 days after administration of 1 × 10⁸ or 1 × 10⁷ sporocysts. Microscopic changes consist of congestion, erosion of enterocytes, villous atrophy, and cryptitis. No disease was seen in 10- to 13-week-old kittens inoculated with up to 10⁷ oocysts. Cats develop immunity to infection, but it is not complete because some oocysts are shed after challenge (38).

*I. felis* infections. *I. felis* develops in enterocytes in the small intestine and occasionally the cecum (161). Three structural types of asexual stages are recognized. The first asexual division is by endodyogeny. The prepatent period is 7 to 11 days, and oocysts are excreted for about 11 days.

Experimental studies indicate that *I. felis* is not pathogenic for cats over 1 month of age (83, 161). Few signs of disease are seen in 6- to 13-week-old cats given 1 × 10⁵ to 1.5 × 10⁵ oocysts. Mild microscopic changes consisting of congestion, erosion of superficial enterocytes, and neutrophil infiltration may be seen. Four-week-old kittens are the most susceptible, and enteritis, emaciation, and death can occur after inoculation of 10⁵ oocysts (1).

Cats develop immunity to *I. felis*, because after infection, they have no or decreased oocyst production when challenged with *I. felis* oocysts. Studies indicate that cats infected naturally with *I. felis* develop lower antibody titers than do those experimentally inoculated with *I. felis* (142). If these cats are challenged with *Toxoplasma gondii*, they will develop an antibody titer to *T. gondii* and demonstrate an anamnestic response to *I. felis* antigen. A 22-kDa peptide on sporozoites is the major *I. felis* protein antigen recognized by immune feline serum (143). Peptides of 22, 45, 58, and 62 kDa on *T. gondii* tachyzoites or...
sporozoites are recognized by *I. felis* immune feline serum. Absorption of *I. felis* immune serum with these *T. gondii* stages removes reactivity of the 45-, 58-, and 62-kDa peptides, implying that the 22-kDa peptide is specific to *I. felis*.

*I. felis* and *T. gondii* have evolved an unusual relationship in the feline definitive host (20, 33, 37). Cats that have previously recovered from a *T. gondii* infection will reexcrete *T. gondii* oocysts if they receive a primary challenge with *I. felis* oocysts. Cats that have a primary *I. felis* infection followed by a primary *T. gondii* infection develop strong immunity to *T. gondii* and will not reexcrete *T. gondii* oocysts if challenged with *I. felis* oocysts. The biological significance or mechanism of this relationship is unknown.

### Diagnosis

Fecal flotation with Sheather’s sugar solution is the recommended method. It is important to examine stools for bacterial and viral agents that cause disease in these animals because coccidiosis is usually asymptomatic. Dogs are coprophagic and often will have oocysts from other animal feces in their samples. It is important to recognize these pseudoparasites. The most common of these are *Eimeria* species from ruminants, rabbits, or rodents. These oocysts will not be in the two-celled stage as is common for *Isospora* species. They often will have ornamentalations, such as micropyle caps or dark thick walls, that are not found on *Isospora* oocysts. *Isospora* oocysts that contain sporocysts with Stieda bodies are also pseudoparasites. Cats may also have coccidial pseudoparasites in their feces from the ingestion of prey.

### Treatment

Sulfadimethoxine given at 50 mg/kg orally once a day for 10 to 14 days will eliminate oocyst excretion in most dogs and cats (104, 191). The combination of ormetoprim (11 mg/kg) and sulfadimethoxine (55 mg/kg) given orally for up to 25 days has been used effectively in dogs (45). Amprolium given orally once a day at 300 to 400 mg/kg for 5 days or 110 to 220 mg/kg for 7 to 12 days is effective in treating coccidiosis in dogs. Other agents such as furazolidone, quinacrine, and metronidazole probably are of little clinical value.

### ISOSPORA SUIS INFECTIONS OF PIGS

The actual number of valid species of coccidia that infect swine is unknown because most are known only from the sporulated-oocyst stage. Levine and Ivens list 13 named species of *Eimeria* and 3 species of *Isospora* isolated from swine (97). *I. suis*, *I. almataensis*, and *I. neyrai* are the species of *Isospora* isolated from swine. *I. almataensis* and *I. neyrai* are known only from oocysts in the feces. *I. almataensis* is most probably a combination of a bird *Isospora* sp. and *I. suis*.

Biester and Murray described *I. suis* from pigs in 1934 (10–12). However, it was not recognized as a major cause of disease in nursing piglets until the early 1970s (167, 171). This probably reflects the modernization of the swine production industry and the use of confinement facilities for the farrowing (birthing) of piglets.

### Clinical Signs and Pathogenicity

Coccidiosis in pigs is a severe disease of nursing piglets (52, 168). *I. suis* is the cause of neonatal porcine coccidiosis (167). There are no reports of coccidiosis caused by *I. almataensis* or *I. neyrai*. *Eimeria* species do not cause clinical coccidiosis in nursing pigs (106). Neonatal porcine coccidiosis caused by *I. suis* is ubiquitous where pigs are farrowed in confinement (21, 25, 31, 51, 99, 139, 152, 156, 157) and is responsible for 15 to 20% of the cases of piglet diarrhea seen at diagnostic laboratories in the United States, Canada, and other countries. Outbreaks of coccidiosis occur year-round. *I. suis* can be seen in nursing piglets suffering from other neonatal diarrheal diseases, and it increases the severity of disease caused by these agents (135, 150, 151, 171).

Infected piglets develop diarrhea at 7 to 14 days of age. The diarrhea is yellowish to gray and initially pasty but becomes fluid after 2 to 3 days; blood is never present if *I. suis* is the only infectious agent. If blood is present, other agents are involved as primary or copathogens. Piglets become covered with diarrheic feces, causing them to stay damp and smell like soured milk. They become lethargic but continue to nurse. Infections fail to respond to commonly used antibiotics. Piglets within a litter and all litters in the farrowing house are not equally affected by coccidiosis. Morbidity is high, and mortality is moderate. Microscopic changes consist of villous atrophy, villous fusion, necrotic enteritis, and crypt hyperplasia (52, 74, 173, 183). Experimental studies indicate that the development of clinical disease and microscopic lesions are dependent upon the number of oocysts inoculated and the age at which piglets are inoculated (13, 86, 112, 153, 172, 173). Doses of 5 × 10⁴ oocysts or less generally produce diarrhea but no mortality in young (1- to 3-day-old) piglets, doses of 7 × 10⁴ to 3 × 10⁵ oocysts cause low to moderate mortality, and doses of 4 × 10⁶ or greater cause high mortality in young piglets. Weight gains of infected piglets are depressed (111).

There is some evidence that *I. suis* may cause postweaning diarrhea in 5- to 6-week-old piglets (138), with diarrhea beginning 4 to 7 days after the piglets are weaned. Morbidity is 80 to 90%, but mortality is very low.

Endogenous stages are found throughout the small intestine and occasionally in the cecum and colon (73, 113, 123, 173). Parasite densities are highest in the jejenum and ileum. Developmental stages are found in enterocytes. Two types of meronts are produced (113). Type 1 meronts are binucleate and divide by endodyogeny (122, 123), whereas type 2 meronts are multinucleate. Both types of meronts are motile and can actively exit and penetrate host cells (110). The prepatent period is 4 to 5 days, and the patent period is 2 weeks or longer. Several peaks in oocyst numbers can occur during the patent period (21, 73, 184). Extraintestinal stages of *I. suis* have not been found by microscopic examination of tissues from infected piglets or in experimentally inoculated mice (73, 123, 148, 170). Oral feeding of mouse or pig tissues was inconclusive in one study (170). Transmission occurred following intraperitoneal inoculation of intestinal lymph node homogenates or homogenates of pooled spleen and liver from pigs inoculated 1 or 2 days previously with large numbers (5 × 10⁷ and 1 × 10⁷ oocysts, respectively) of *I. suis* oocysts (73). The prepatent period was 10 to 12 days in the recipient pigs.

The role of viral and bacterial copathogens with *I. suis* has been examined experimentally (5, 185). The responses of nontoxic and conventional pigs to *I. suis* and rotavirus coinfection are similar (185). The degree of observed clinical disease is more severe when the pathogens are administered concur rently than when either is given singly. Both the virus and the parasite develop preferentially in the enterocytes of the central and distal portion of the villi in the small intestine, and competition for a suitable host cell is believed to be the cause of the observed increase in clinical disease and microscopic lesions. An established *I. suis* infection will interfere with the establishment of a *Salmonella typhimurium* infection (5). The increased gut motility and destruction of host cells probably
interfere with the ability of the bacterium to colonize the intestinal mucosa.

**Immunity**

Piglets that recover from *I. suis* infection exhibit a strong degree of resistance to reinfection. No clinical signs develop after challenge, and few or no oocysts are excreted in the feces (174). Colostral antibodies against *I. suis* do not protect piglets from developing clinical coccidiosis (177). Antibody levels in serum peak about 1 week after primary infection, and a secondary antibody response occurs following challenge infection. Serum antibodies against *I. suis* do not recognize sporozoites of *Eimeria debliecki*, *E. neodebliecki*, *E. scabra*, or *E. porci* in an indirect fluorescent-antibody test. Lymphocyte migration inhibition responses of pigs that are immune to *I. suis* are significantly lower than those of controls when soluble or particulate *I. suis* sporozoite antigens are used. Polymorphonuclear leukocyte (PMN) chemotactic factors were generated by lymphocytes from piglets inoculated with *I. suis* and incubated with soluble or particulate sporozoite antigens. Lymphocytes from control pigs did not produce chemotactic factors for PMNs after incubation with *I. suis* sporozoite antigens, and the antigens alone were not chemotactic for PMNs.

**Epidemiology**

The epidemiology of neonatal porcine isosporiasis is puzzling. Sows are often infected with *Eimeria* species, but the prevalence of *I. suis* is usually less than 5% (50, 69, 114, 182). The sow is a logical source of infection for newborn piglets, but studies conducted in the United States have failed to demonstrate *I. suis* oocysts in a significant number of sows (50, 114, 168). *I. suis* oocysts were not found in the feces of 77 sows examined from 7 farms with a problem of neonatal coccidiosis caused by *I. suis*, and only 1 of 172 sows examined from 27 farms without a history of neonatal coccidiosis was positive (114). *Eimeria* oocysts were found in 91% of these sows. In another study, sows from two farms with neonatal coccidiosis in piglets were examined on a daily basis for about 1 week prior to farrowing, at farrowing, and for about 2 weeks postfarrowing (168). *I. suis* oocysts were not found in these sows; however, piglets nursing from these sows developed coccidiosis and excreted *I. suis* oocysts at 4 to 8 days of age. Microscopic examination of milk samples and placental from these sows were negative for parasites. Once *I. suis* is established on a farm, it is probably maintained by infection of piglets from the contaminated farrowing crate.

**Diagnosis**

Diagnosis is based on a clinical history suggestive of coccidiosis and the demonstration of *I. suis* oocysts in fecal samples or the demonstration of developmental stages in mucosal smears or histological sections obtained from necropsy specimens (101). Samples for oocyst identification should be taken from pigs that have had diarrhea for 2 days or more because clinical signs often appear before oocysts are excreted in the feces (173). Pasty fecal samples are likely to contain more oocysts than are liquid samples. Fecal fat makes identification of oocysts in Sheather’s sugar flotation preparations difficult. A solution of saturated sodium chloride and glucose has been recommended as an alternative flotation medium (79).

The use of mucosal imprints stained with any Giemsa-type stain is a reliable method for diagnosing porcine coccidiosis (101). Imprints should be taken from the jejunum or ileum because these are the sites where parasite densities are highest.

The presence of paired merozoites indicative of division by endodyogeny is characteristic for *I. suis* in pigs (101). Histological sections taken from the jejunum or ileum also contain developmental stages in the enterocytes.

**Treatment and Control**

Anticoccidial treatment of piglets has generally proven unrewarding. Nursing piglets do not eat or drink enough to make antibiotics added to feed or water useful. Catching each piglet for dosing is time-consuming and labor-intensive and probably practical only on small farms. Controlled studies indicate that amprolium, monensin, and furazolidone are not effective in preventing coccidiosis in nursing piglets (29, 67). Toltrazuril does show promise as an effective means of preventing coccidiosis in nursing piglets (30). When 20 to 30 mg of toltrazuril/kg was given orally as a single dose to 3- to 6-day-old piglets, coccidiosis was reduced from 71 to 22%. The severity of diarrhea and oocyst excretion was reduced in toltrazuril-treated piglets.

Lasalocid and halofuginone have been evaluated in early-weaned pigs experimentally infected with *I. suis* (118, 124). Lasalocid given at 150 mg/kg of feed prevented weight loss in pigs but did not prevent oocyst excretion. These pigs developed strong immunity to reinfection. Halofuginone given at 6 mg/kg of feed inhibited oocyst production but caused reduced weight gains due to poor feed intake. The halofuginone-treated pigs did not develop strong immunity to challenge infection.

Improved sanitation is the best means of controlling neonatal coccidiosis (50). Feeding anticoccidial agents to sows is not recommended because they are not the source of fecal oocysts for their nursing piglets. Commercially available disinfectants do not inhibit the development of *I. suis* oocysts when used at the concentrations recommended by the manufacturers (169). Once the oocysts sporulate, they are even more resistant to disinfectants. Steam cleaning is effective in killing sporulated and unsporulated oocysts and is an effective means of decreasing piglet exposure to infective *I. suis* oocysts. Additional preventive measures are for farm workers to limit their access to infected piglets to prevent crate-to-crate spread of infection via their boots. Also, flies and other insects should be controlled to prevent them from mechanically carrying oocysts from crate to crate. Supportive measures such as providing water or electrolyte solutions in piglet waters may be of value in preventing dehydration in clinically infected piglets.

**FUTURE DIRECTIONS**

Future studies on human *I. belli* infections need to examine the latent tissue cyst stage of the parasite. It is most probably responsible for the recrudescence of clinical disease which is observed in AIDS and other patients. It is obvious that the tissue cyst stage is not killed by treatment with TMP-SMX. An in vitro culture system needs to be developed to examine potential anticoccidial agents against *I. belli* and to study tissue cyst biology. Experimental inoculation of rodents with *I. belli* oocysts needs to be examined to determine whether latent infections develop in these animals. If rodents are found to be paratenic hosts, potentially other food animals may also be capable of transmitting the infection.

Additional studies on dog and cat *Isospora* spp. should be conducted to determine if these coccidia are truly pathogenic in them. Studies with neonatal porcine coccidiosis should focus on identifying effective treatments and vaccines.

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