Parasitology

cambridge.org/par

Endogenous development of *Cystoisospora belli* in intestinal and biliary epithelium of humans

J. P. Dubey[1], Kimberley J. Evason[2] and Zenta Walther[3]

[1]United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Beltsville, Maryland, 20705-2350, USA; [2]Department of Pathology and Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112, USA and [3]Department of Pathology, Yale University School of Medicine, New Haven, CT 06520-8023, USA

Research Article

Cite this article: Dubey JP, Evason KJ, Walther Z (2019). Endogenous development of *Cystoisospora belli* in intestinal and biliary epithelium of humans. *Parasitology* **146**, 865–872. https://doi.org/10.1017/S003118201900012X

Received: 14 January 2019
Revised: 23 January 2019
Accepted: 24 January 2019
First published online: 12 March 2019

Key words:
Coccidia; *Cystoisospora* (Isospora) *belli*; gamonts; meronts; merozoites; oocysts; protozoa; schizonts

Author for correspondence:
J. P. Dubey, E-mail: jitender.dubey@ars.usda.gov

Abstract

Cystoisospora (*Isospora*) *belli* is a coccidian parasite of humans. It can cause serious digestive disorders involving infection of intestines, biliary tract and gallbladder, especially in those with depressed immunity. It has a direct fecal–oral transmission cycle. After ingestion of sporulated oocysts, the parasite multiplies asexually and sexually within host epithelial cells, resulting in unsporulated oocysts that are excreted in feces. The details of asexual and sexual stages are not known and certain inclusions in epithelial cells in biopsy samples have been erroneously identified recently as *C. belli*. Here, we provide details of developmental stages of *C. belli* in two patients, in duodenal biopsy of one and biliary epithelium of the other. Immature and mature asexual stages (schizonts/meronts) were seen in epithelial cells. The merozoites were seen singly, in pairs and in groups in single parasitophorous vacuole (pv) in host cytoplasm. Immature and mature meronts were seen together in the same pv; up to eight nuclei were seen in meronts that retained elongated crescent shape; round multinucleated schizonts, seen in other coccidians, were not found. Meronts were up to 25 μm long and contained up to ten merozoites that were 8–11 μm long. The merozoites and meronts contained PAS-positive granules. Microgamonts (male) contained up to 30 nuclei that were arranged at the periphery and had condensed chromatin; 1–3 PAS-positive, eosinophilic, residual bodies were left when microgametes were formed. The microgametes were 4 μm long and PAS-negative. All stages of macrogamonts, including oocysts were PAS-positive. The detailed description of the life cycle stages of *C. belli* reported here should facilitate in histopathologic diagnosis of this parasite.

Introduction

Cystoisospora belli is a coccidian parasite of humans. Originally named by Wenyon (1923) as *Isospora belli* for its bell-shaped oocysts; it was transferred to the genus *Cystoisospora* (Barta *et al.*, 2005). The parasite is specific to humans and attempts to infect non-human primates, livestock, rodents and other animals with *C. belli* were unsuccessful (Jeffery, 1956). Experimental infections in human volunteers and in laboratory acquired infections revealed that it has a direct fecal–oral transmission cycle and can cause serious illness in humans (Matsubayashi and Nozawa, 1948; Jeffery, 1956; Ferreira *et al.*, 1962; Henderson *et al.*, 1963). The prepatent period (first day of oocyst excretion in feces) is 9–17 days. Hundreds of cases of intestinal infections have been reported in both immunocompetent and immunocompromised humans (Faust *et al.*, 1961; Jarpa Gana, 1966; Legua and Seas, 2013). In addition to enteritis, *C. belli* can cause infections of the biliary tract and gallbladder (Benator *et al.*, 1994; Agholi *et al.*, 2016).

Fragmentary information on endogenous stages was obtained by examination of human biopsy tissues from small intestine (Niedmann, 1963; Brandborg *et al.*, 1970; Henry *et al.*, 1974; Trier *et al.*, 1974; Liebman *et al.*, 1980; Veldhuyzen van Zanten *et al.*, 1984; Modigliani *et al.*, 1985; Restrepo *et al.*, 1987; Peng and Tsai, 1991; Benator *et al.*, 1994; Comin and Santucci, 1994; Michiels *et al.*, 1994; Hamour *et al.*, 1997; Bialek *et al.*, 2001; Field, 2002; Jongwutiwes *et al.*, 2002; Sasaki *et al.*, 2004; Walther and Topazian, 2009; Murphy *et al.*, 2011; Rao *et al.*, 2012; Kim *et al.*, 2013; Agholi *et al.*, 2016; Lai *et al.*, 2016; Martelli and Lee, 2016; Swanson *et al.*, 2018), bile ducts or gallbladder (Benator *et al.*, 1994; Lagrange-Xélot *et al.*, 2008; Walther and Topazian, 2009; Agholi *et al.*, 2016; Martelli and Lee, 2016). Meronts, microgamonts, macrogamonts and unsporulated oocysts were seen in enterocytes of small intestine and in biliary epithelium. Histopathologic examination of tissues obtained post-mortem confirmed that asexual and sexual stages of *C. belli* are confined to the intestines, bile ducts and gallbladder epithelium (Brandborg *et al.*, 1970; Restrepo *et al.*, 1987; Benator *et al.*, 1994; Michiels *et al.*, 1994; Frenkel *et al.*, 2003). Restrepo *et al.* (1987) mentioned finding of schizonts and gamonts also in large intestine but did not provide any details. Additionally, monozoic tissue cysts (thought to be encysted sporozoites) were found in the lamina propria of small and large intestine, lymph nodes, spleen and liver of immunosuppressed patients (Restrepo *et al.*, 1987; Comin and Santucci, 1994; Michiels *et al.*, 1994; Lindsay *et al.*, 1997; Velásquez *et al.*, 2001, 2011; Frenkel *et al.*, 2003; Meamar *et al.*, 2009;

CAMBRIDGE UNIVERSITY PRESS

Swanson et al., 2018). Parasitaemia has been reported in one patient (Velásquez et al., 2016). After a review of findings in these reports, it became evident that details of the development of *C. belli* stages are lacking.

The diagnosis of *C. belli* can be made by fecal examination for its characteristic 20–37 μm long bell-shaped oocysts, and by histological and molecular examination of biopsy specimens. Fecal examination is the simplest but can be problematic, because the oocysts are excreted intermittently, often in small numbers, and can be absent during acute infection. Histological examination of biopsy of small intestine, bile duct and gallbladder can confirm diagnosis depending on the size of the tissue sample and the density of parasites. However, bile is a cytolytic agent (Tatum, 1916), which can lead to gallbladder epithelial inclusions mimicking *C. belli* schizonts and gamonts (Lai et al., 2016; Martelli and Lee, 2016; Akateh et al., 2018) and potential misdiagnosis of infection (Swanson et al., 2018).

The objective of the current paper is to describe development of *C. belli* in intestine and biliary tract of two patients. The detailed description of the life cycle stages of *C. belli* reported here should facilitate in histopathologic diagnosis of this parasite.

Materials and methods

Case 1 (common bile duct)

Biopsy specimens of the common bile duct from the 42-year-old patient reported by Walther and Topazian (2009) were studied here. Three 3 × 1 mm biopsy fragments were embedded in a paraffin block and multiple sections were mounted on a total of nine histological slides; the block was subsequently exhausted for molecular studies, with no tissue remaining. Nine sections were examined microscopically for parasite stages after staining with haematoxylin and eosin (HE). Retrospectively, one slide stained with HE was re-stained with periodic acid Schiff reaction (PAS), counter stained with haematoxylin (PASH).

Case 2 (duodenum)

Five 2–3 × 1 mm pieces of duodenal biopsy from a 23-year-old patient with acquired immune deficiency syndrome (AIDS) and diarrhoea (Swanson et al., 2018) were embedded in a paraffin block. Seven sections from this block were studied for the present investigation. The sections were stained with HE, PASH or trichrome stain.

All host tissue from both cases was examined by one of us (J.P.D.) at 1000× magnification in an Olympus AX 70 microscope and stages photographed using a DP73 camera.

Results

In both cases, asexual and sexual stages of *C. belli* were present mainly in epithelial cells of surface epithelium and rarely in crypt epithelial cells. The parasites were located above or below the host cell nucleus and the host cell nucleus was indented but not hypertrophied. All stages were in host cytoplasm within a parasitophorous vacuole (pv).

To avoid confusion and to document variability in PAS staining characteristics, both cases are described separately below.

Case 1 (common bile duct)

The density of parasites varied; in some microscopic fields, many parasites were seen (Fig. 1A) while most had only a few organisms. Some infected host cells were bulging into the lumen. Both immature and mature meronts were recognized. Immature meronts were elongated to pear-shaped. Meronts were up to 25 × 18 μm and contained up to ten merozoites. Merozoites were present singly, in pairs and in groups (Fig. 1). Merozoites were approximately 8–12 × 2–4 μm. In HE-stained sections, individual merozoites were elongated with a pointed (conoidal) end, a central nucleus, had a few lightly stained areas, and measured 10–11 × 3 μm (Fig. 1A). Figure 1C shows a meront containing two merozoite-like structures that are 11 × 4 μm. The nuclei in larger meronts were not well defined. Merozoites and meronts contained PAS-positive granules; the intensity of staining varied. In most merozoites, PAS-positive granules were concentrated at the polar ends of merozoites (Fig. 1D and F).

Microgamonts were rounded, oval or elongated (Fig. 2A–D). The earliest microgamont was about 6 μm in diameter and contained four nuclei. With maturation of the microgamonts, the nuclei moved peripherally and a small PAS-positive residual body was visible. Figure 2C shows a microgamont with 30 nuclei and a very small PAS-positive body. Figure 2D shows an 18 × 10 μm microgamont with at least 16 nuclei and three PAS-positive residual bodies. Microgametes were about 4 μm long (Fig. 2E) and PAS-negative.

Macrogamonts could be distinguished from meronts and microgamonts by the presence of a single nucleus with a large nucleolus. In sections stained with HE, dusting of granules was seen but wall forming bodies (WFB) were not recognized. The earliest macrogamonts were about 6 μm in diameter (Fig. 2F and G). All macrogamonts were intensely PAS-positive (Fig. 2G). As the macrogamonts matured, they became elongated. Only a few oocysts were seen and they contained an intensely PAS-positive sporont and a very thin oocyst wall (Fig. 2H and I).

Case 2 (duodenum)

The youngest meronts were crescent-shaped, 11–15 × 3–4 μm and contained two to four nuclei; their ends were either round or pointed (Fig. 3A and B). In more advanced meronts up to eight nuclei were recognized (Fig. 3E–G). Figure 3G shows an elongated 20 × 6 μm meront with a pointed conical end and a distal rounded end; it appears to have more than eight indistinct nuclei. The pointed end is stained differently than the rest of the meront. Figure 3F shows a 15 × 6 μm meront with eight nuclei. Figure 3C and D shows meronts with paired developing crescent-shaped structures. Figure 3J shows a meront containing both mature merozoites and in division. Individual merozoites containing single nucleus were 8–11 × 2–3 μm (Fig. 3H–J). There was no residual body in meronts; sometimes merozoite in cross-section appeared like a residual body (Fig. 3H).

Both immature and mature gamonts were identified. The youngest microgamont was 9 × 8 μm and appeared to have four nuclei. In early microgamonts, the nuclei filled the entire gamont (Fig. 4A). With maturation of the microgamont, the nuclear chromatin condensed, and the nuclei moved to the periphery of the gamont (Fig. 4B and C). A small PAS-positive residual body was first recognized in a 9 × 7 μm microgamont containing 14 peripherally arranged nuclei (Fig. 4B). Mature microgamonts contained one or more large eosinophilic, PAS-positive residual bodies and peripherally arranged 4 μm long PAS-negative microgametes (Fig. 4D).

All stages of macrogamonts were PAS-positive. Early macrogamonts were round to oval and contained a central single nucleus with a large nucleolus. With maturation, the macrogamonts became elongated with pointed or rounded ends (Fig. 4E–H). In HE-stained sections, the nucleus was eosinophilic with darkly stained area at one end (Fig. 4F). The cytoplasm contained variably stained granules. Mature macrogamonts were up to 21 μm long. Only one oocyst with partial wall was seen (Fig. 4I).

Tissue cysts were seen in the lamina propria of the duodenum. They were approximately 14 × 7 μm and contained an 8 × 4 μm sporozoite. The sporozoites contained a central nucleus and few

https://doi.org/10.1017/S003118201900012X Published online by Cambridge University Press

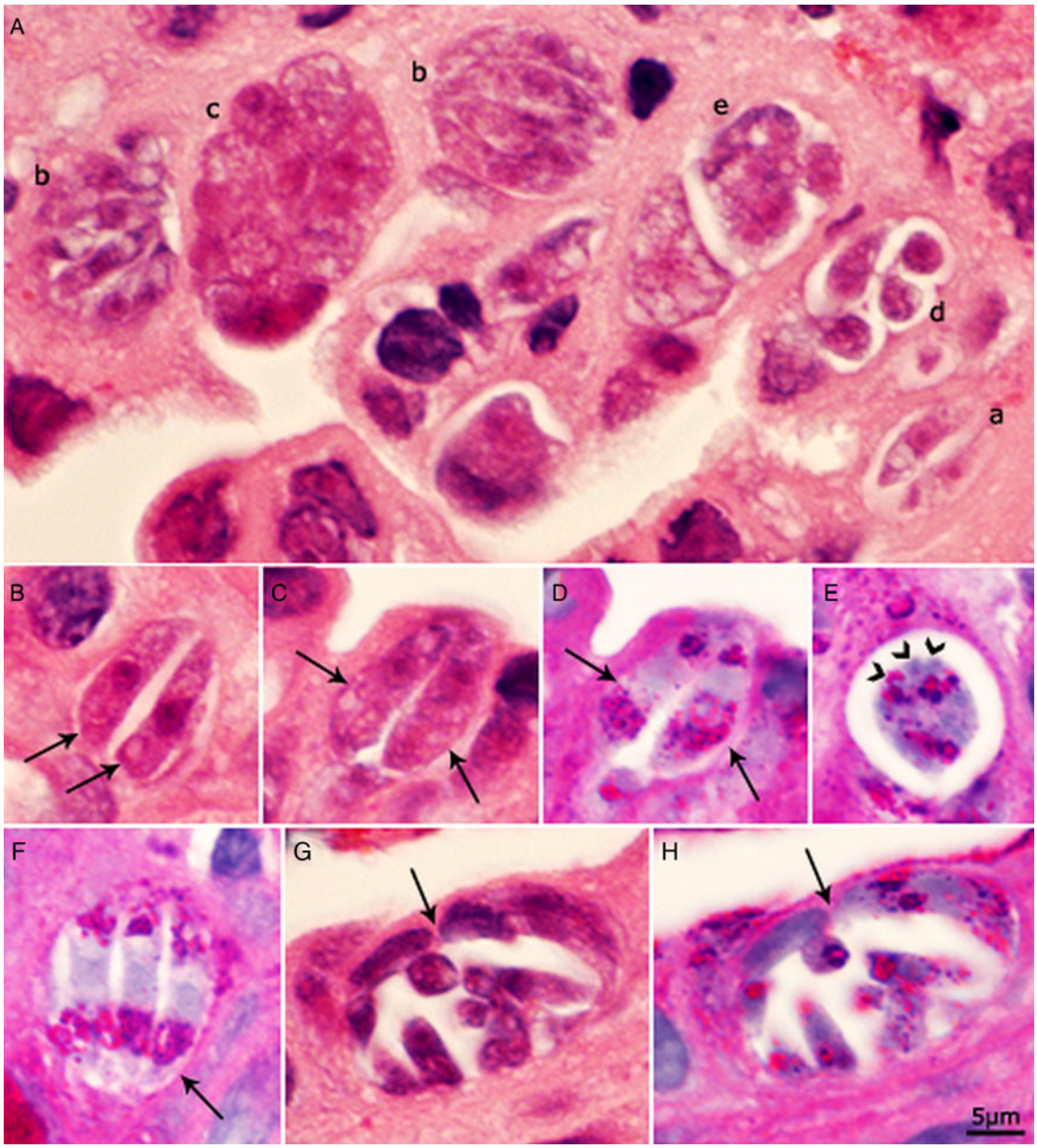

Fig. 1. Asexual stages of *C. belli* in histological sections of bile duct of a patient. Bar applies to all parts. A, B, C and G = HE stain; D, E, F and H = PAS counter stained with haematoxylin. The luminal side of sections is on the top. (A) Several meronts in one microscopic field. (a) Paired merozoites in one pv. (b) Meront with four or more merozoites completely filling the pv. (c) Meront containing developing merozoites. The organisms are cut in cross-section; the nuclei appear larger than in merozoites. (d) Four merozoites in a pv with spaces between merozoites. (e) Immature meront. (B) Paired crescent-shaped merozoites. (C, D) The same paired organisms/meronts after staining with HE (C) and PAS (D). Note the polar PAS-positivity. The parasite nuclei are masked by PAS staining. (E) Meront with merozoites budding (arrowheads). Note PAS-positivity. (F) Meront (arrow) with three merozoites with polar PAS-positivity. (G, H) The same mature meront (arrow) after staining with HE (G) and PASH (H).

faintly stained PAS-positive granules. These tissue cysts were structurally like those described previously (Restrepo *et al.*, 1987).

Discussion

In the current study, the development of asexual and sexual stages of *C. belli* is described and asexual stages were called meronts instead of schizonts. Prior to this description, *C. belli* was thought to follow the life cycle of *Eimeria* species of poultry and livestock (Trier *et al.*, 1974). In a typical *Eimeria* life cycle, the asexual stages are called schizonts and the parasite nucleus divides into six or more nuclei before merozoites are formed; in some *Eimeria* spp. thousands of nuclei are produced before merozoite formation (Levine, 1973). Schizogony consists of a series of generations of merozoite formation in different cells that are morphologically different in each generation and occur at different time periods [up to six distinct generations are recognized in some *Eimeria* species (Levine, 1973)]. Dubey and Frenkel (1972) first proposed the term 'type' for intestinal stages of *Toxoplasma gondii* because there was a profuse asexual multiplication of meront types long after gametogony in feline enterocytes; asexual generations could not be determined. Subsequently, it was found that *Isospora* (now *Cystoisospora*) of dogs and cats followed the same pattern as in *T. gondii* (Dubey, 2018; Dubey and Lindsay, 2019). In *Cystoisospora* species, the parasites divide by endodyogeny (division into two), by fission, and multiple endodyogeny; typical multinucleated round schizonts are absent. The parasite can have multiple replications without leaving the host cell. Thus, immature meronts occur along with mature merozoites in the same vacuole. These stages were found here in *C. belli* for

https://doi.org/10.1017/S003118201900012X Published online by Cambridge University Press

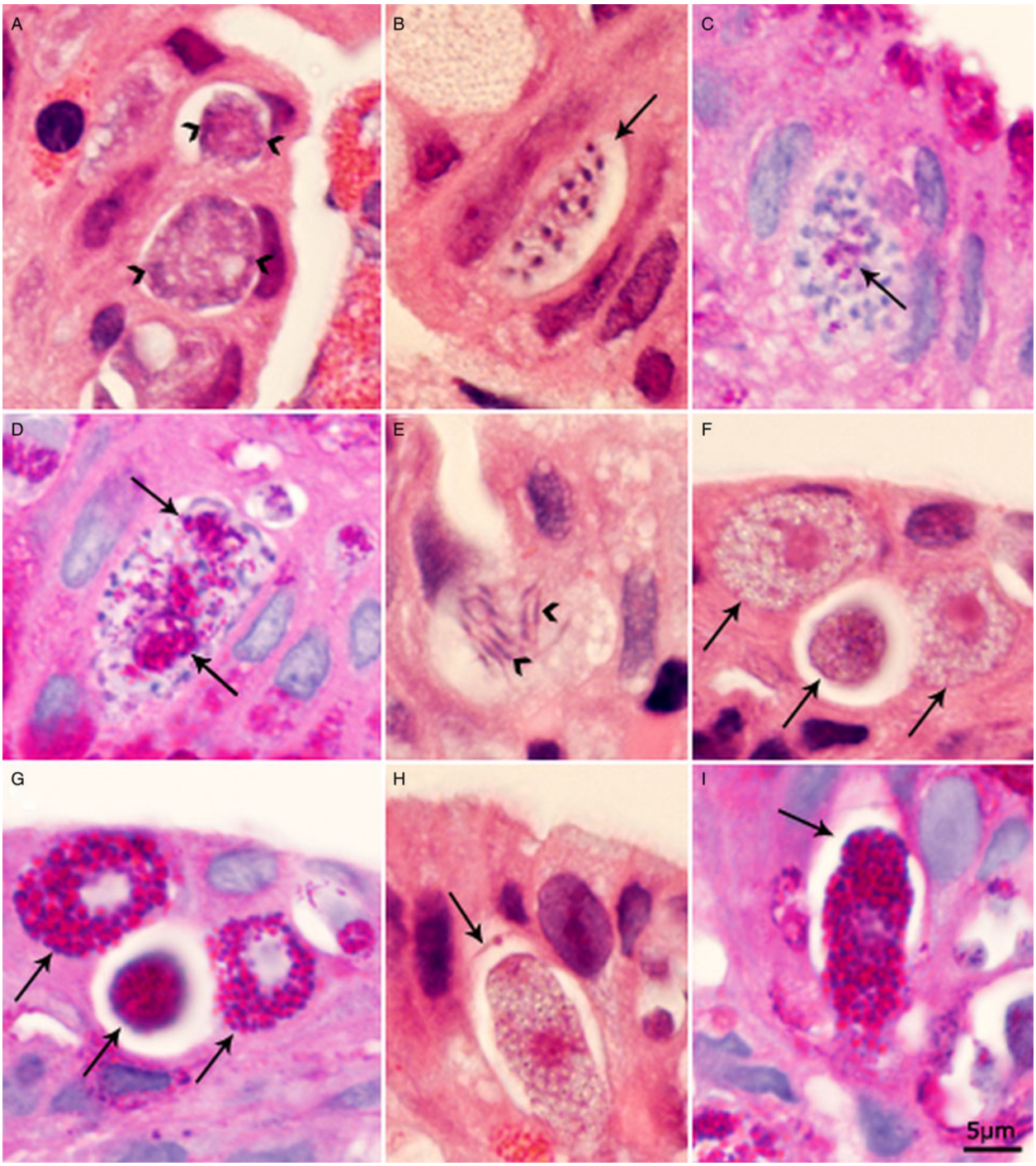

Fig. 2. Sexual stages of *C. belli* in histological sections of biliary epithelium. Bar applies to all parts. A, B, E, F and H = HE stain; C, D, G and I = PAS counter stained with haematoxylin. (A) Two multinucleated microgamonts with peripheral nuclei (arrowheads); the nuclear structure is not clear. (B) An elongated microgamont (arrow) with several peripheral nuclei with condensed chromatin. (C) A microgamont with many nuclei and a small PAS-positive body. (D) A microgamont with two large residual bodies (arrows) and several microgametes. (E) Microgametes (arrowheads). (F, G) The three same young macrogamonts (arrows) after staining with HE (F) and PASH (G). Note, intensely stained PAS-positive granules in cytoplasm that do not stain with HE. (H, I) Same macrogamont with oocyst wall (arrow) after staining with HE (H) and after staining with PASH (I). The gamont is intensely PAS-positive.

the first time. Therefore, the term type is more applicable than the generation/schizont. The term meront also is not specific with respect to division of the parasite; it applies both to the product of endodyogeny or schizogony. Because the ultrastructural details of mode of division of most *Cystoisospora* spp. are largely unknown, we used the term meront in the current paper.

In the cases studied here, the diagnosis of *C. belli* had been confirmed previously by polymerase chain reaction (Walther and Topazian, 2009; Swanson *et al.*, 2018). In both cases, there was no histologic evidence of *Cyclospora cayetanensis*, the other coccidian found in enterocytes of humans (Ortega *et al.*, 1997). Compared with *C. belli*, the oocysts of *C. cayetanensis* are tiny (8–10 μm). Endogenous stages of *C. cayetanensis* are also structurally different from *C. belli*. Two types of *C. cayetanensis* meronts were reported (Ortega *et al.*, 1997). Type I meronts contained 8–12 tiny (3–4 × 0.5 μm) mature merozoites. Type II meronts contained four large slender (12–15 × 0.7–0.8 μm) merozoites.

Trier *et al.* (1974) first illustrated meronts, microgamonts, macrogamonts and oocysts of *C. belli* by transmission electron microscopy (TEM) but did not elaborate on details of the observed

https://doi.org/10.1017/S003118201900012X Published online by Cambridge University Press

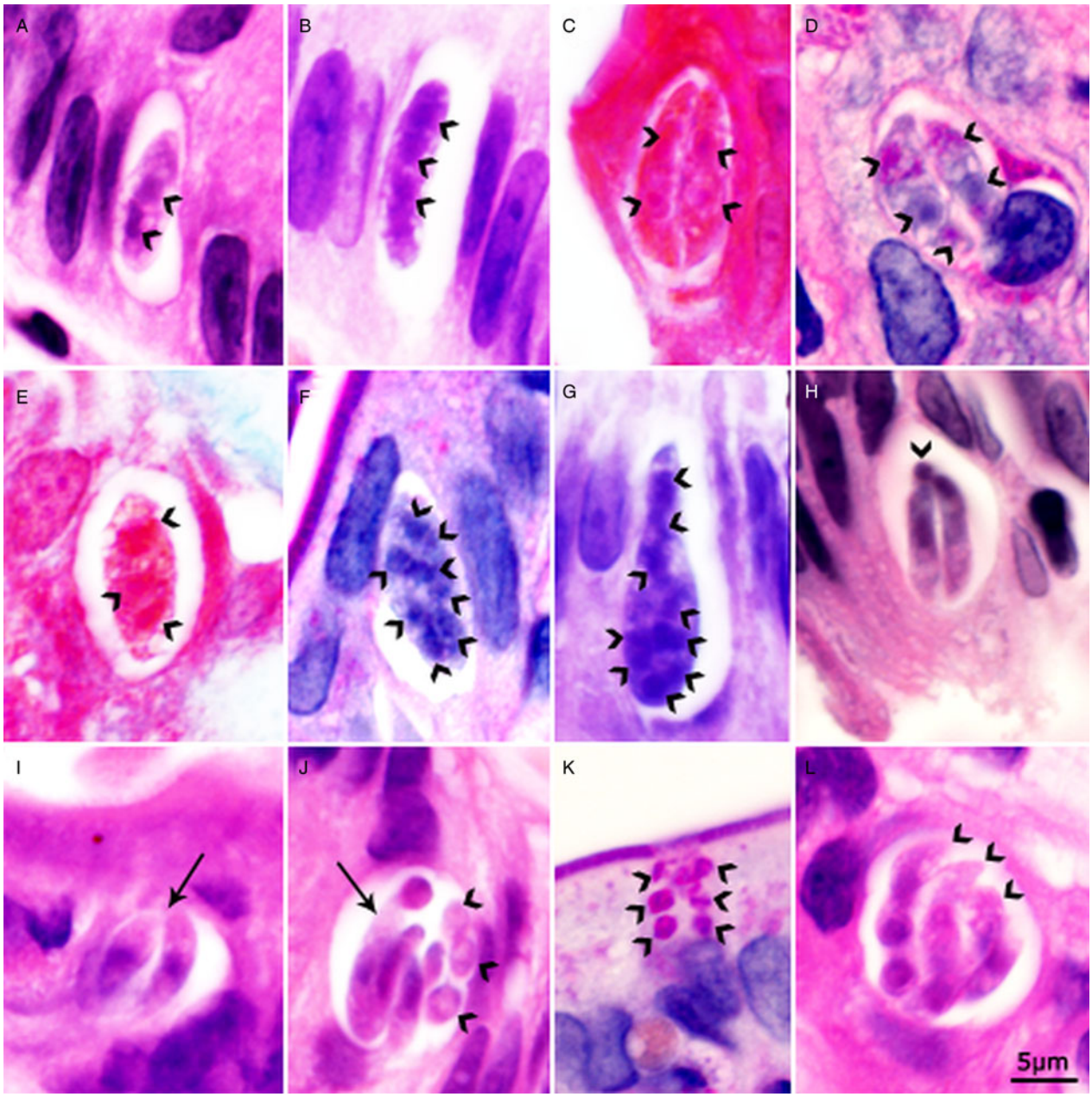

Fig. 3. Asexual stages of *C. belli* in histological sections of duodenum of a patient. Bar applies to all parts. A, B, G, H, I, J and L = HE stain; C and E = trichrome stain; D, F and K = PAS counter stained with haematoxylin. The luminal side of sections is on the top. (A) Elongated meront with dividing nucleus (arrowheads). (B) Elongated meront with three nuclei (arrowheads). Note both end of the meront are rounded. (C). A pv containing paired crescent-shaped meronts. Note large nuclei (arrowheads). (D) A pv containing paired crescent-shaped meronts. Note large nuclei (arrowheads). (E) Meront with three or more nuclei (arrowheads). (F) Meront with eight nuclei (arrowheads). (G) An elongated meront with a conical (conoidal) and a rounded non-conoidal end. There are more than eight nuclei (arrowheads). (H) A pv containing two slender merozoites and one merozoite in cross-section (arrowhead). (I) A pv with two merozoites that are thicker than merozoites in 1H (arrow). (J) A pv containing an undivided meront (arrow) and five or more merozoites (arrowheads). (K) A pv with six merozoites in cross-section (arrowheads). Note PAS positivity. (L) Mature meront with merozoites (arrowheads).

structures. Figure 6 in their paper shows a meront with four partially sectioned merozoites that contain organelles typically found in coccidian merozoites (Trier *et al.*, 1974). Figure 6A of their paper shows amylopectin granules anterior and posterior to the nucleus of merozoites. These amylopectin granules correspond to PAS-positive granules in merozoites found here. They illustrated several micronemes at the conoidal end that were misinterpreted to be present posterior to the nucleus. They also illustrated an immature macrogamont with numerous electron lucent bodies, which are undoubtedly amylopectin granules (Trier *et al.*, 1974). A few electron dense bodies were present which are probably WFB. Figure 10 of their paper (considered maturing oocyst) is probably a macrogamont; two types of WFB are visible and they are electron dense (Trier *et al.*, 1974). The electron lucent bodies labelled 'L' by them are amylopectin or lipid bodies. Trier *et al.* (1974) correctly labelled microgamonts. Walther and Topazian (2009) also illustrated TEM of a mature microgamont with mature microgametes with flagella.

In conclusion, despite numerous studies mentioning the presence of asexual and sexual stages of *C. belli* in human intestinal and biliary epithelium over the last 50 years no study had successfully described the structural features and modes of development of *C. belli* in humans. The current study achieved that goal using known features of development of intestinal stages of *Cystoisospora* species from dogs, cats and pigs and enteroepithelial stages of *T. gondii* in cats as a foundation to provide a

https://doi.org/10.1017/S003118201900012X Published online by Cambridge University Press

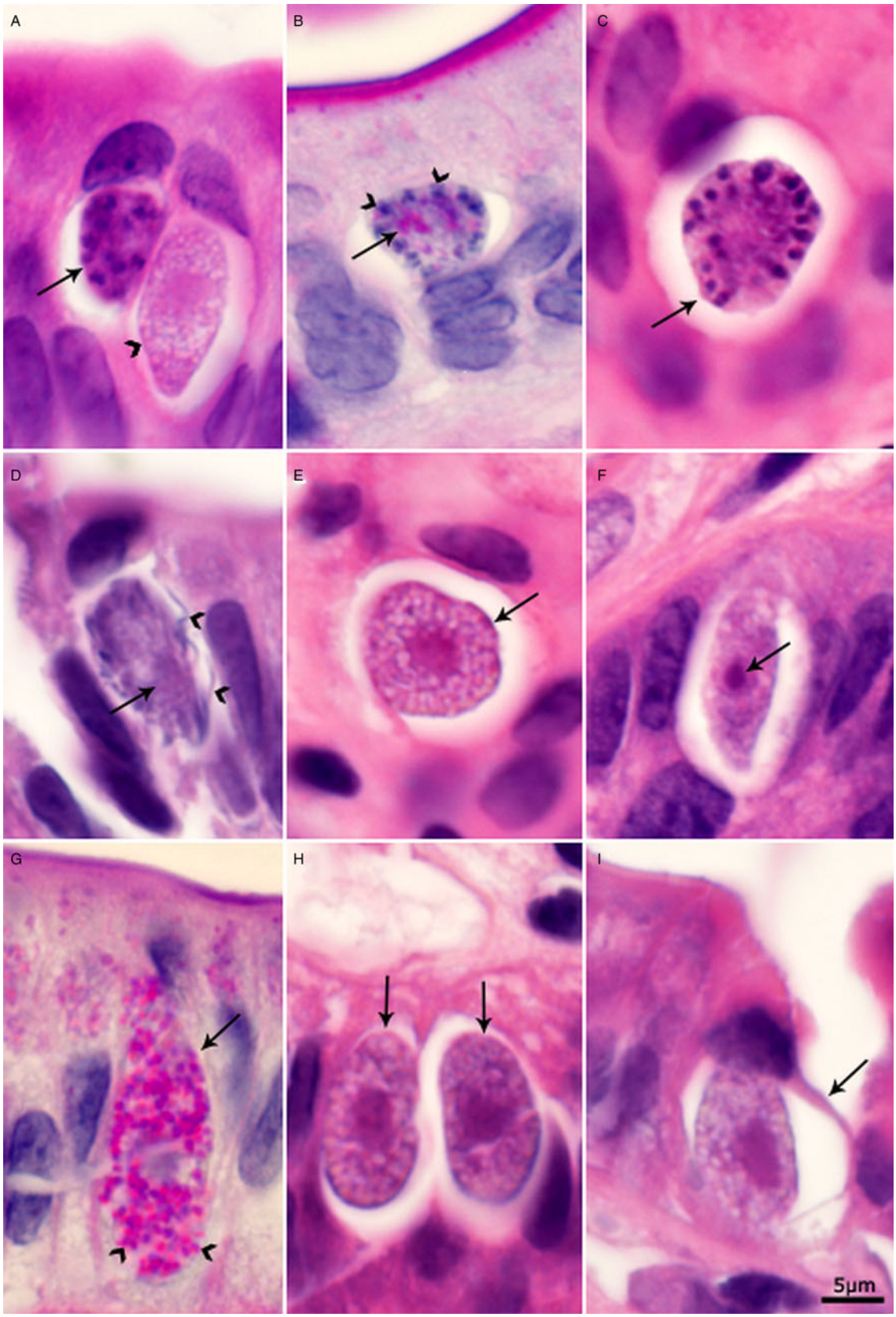

Fig. 4. Sexual stages of *C. belli* in histological sections of duodenum. Bar applies to all parts. A, C, D, E, F, H, and I = HE stain; B and G = PAS counter stained with haematoxylin. The luminal side of sections is on the top. (A) Multinucleated microgamont (arrow) and an elongated macrogamont with a large nucleus (arrowhead). The conical end of the macrogamont is stained darker than the rest of the gamont. Note small granules in the cytoplasm. (B) A microgamont (arrow) with several peripheral nuclei and a small PAS-positive body. (C) A microgamont (arrow) with peripheral nuclei. (D) A mature microgamont with a large residual body (arrow) and microgametes (arrowheads). (E) A macrogamont in cross-section (arrow). Note many small granules/empty spaces. (F) An elongated macrogamont with a single nucleus and a large nucleolus (arrow). Note, both ends of the gamont are pointed. (G) Elongated macrogamont (arrow) with a large nucleus and many PAS-positive granules (arrowheads). (H) Two macrogamonts with large central nucleus (arrows). (I) Macrogamont with developing oocyst wall (arrow).

https://doi.org/10.1017/S003118201900012X Published online by Cambridge University Press

narrative of the endogenous development of *C. belli*. This will provide a useful tool for pathologists and others interested in infectious diseases.

Acknowledgements. We thank Camila Cezar and Fernando Murata for assistance.

Author ORCIDs. J. P. Dubey, 0000-0003-4455-2870

Financial support. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Conflict of interest. None.

Ethical standards. This study used archived material. No experiments were performed.

References

Agholi M, Aliabadi E and Hatam GR (2016) Cystoisosporiasis-related human acalculous cholecystitis: the need for increased awareness. *Polish Journal of Pathology* **67**, 270–276.

Akateh C, Arnold CA, Benissan-Messan D, Michaels A and Black SM (2018) *Cystoisospora belli* gallbladder infection in a liver transplant donor. *Case Reports in Infectious Diseases*. Article ID 3170238.

Barta JR, Schrenzel MD, Carreno R and Rideout BA (2005) The genus *Atoxoplasma* (Garnham 1950) as a junior objective synonym of the genus *Isospora* (Schneider 1881) species infecting birds and resurrection of *Cystoisospora* (Frenkel 1977) as the correct genus for *Isospora* species infecting mammals. *Journal of Parasitology* **91**, 726–727.

Benator DA, French AL, Beaudet LM, Levy CS and Orenstein JM (1994) *Isospora belli* infection associated with acalculous cholecystitis in a patient with AIDS. *Annals of Internal Medicine* **121**, 663–664.

Bialek R, Overkamp D, Rettig I and Knobloch J (2001) Case report: Nitazoxanide treatment failure in chronic isosporiasis. *American Journal of Tropical Medicine and Hygiene* **65**, 94–95.

Brandborg LL, Goldberg SB and Breidenbach WC (1970) Human coccidiosis – a possible cause of malabsorption – the life cycle in small-bowel mucosal biopsies as a diagnostic feature. *The New England Journal of Medicine* **283**, 1306–1313.

Comin CE and Santucci M (1994) Submicroscopic profile of *Isospora belli* enteritis in a patient with acquired immune deficiency syndrome. *Ultrastructural Pathology* **18**, 473–482.

Dubey JP (2018) A review of *Cystoisospora felis* and *C. rivolta*-induced coccidiosis in cats. *Veterinary Parasitology* **263**, 34–48.

Dubey JP and Frenkel JK (1972) Cyst-induced toxoplasmosis in cats. *Journal of Protozoology* **19**, 155–177.

Dubey JP and Lindsay DS (2019) Coccidiosis in dogs – 100 years of progress. *Veterinary Partasitology* **266**, 34–55.

Faust EC, Giraldo LE, Caicedo G and Bonfante R (1961) Human isosporosis in the western hemisphere. *American Journal of Tropical Medicine and Hygiene* **10**, 343–349.

Ferreira LF, Coutinho SG, Argento CA and da Silva JR (1962) Experimental human coccidial enteritis by *Isospora belli* Wenyon, 1923. A study based on the infection of 5 volunteers. *El Hospital; La Revista Interamericana De Hospitales* **62**, 795–804.

Field AS (2002) Light microscopic and electron microscopic diagnosis of gastrointestinal opportunistic infections in HIV-positive patients. *Pathology* **34**, 21–35.

Frenkel JK, de Oliveira Silva MB, Saldanha J, de Silva ML, Correia Filho VD, Barata CH, Lages E, Ramirez LE and Prata A (2003) *Isospora belli* infection: observation of unicellular cysts in mesenteric lymphoid tissues of a Brazilian patient with AIDS and animal inoculation. *Journal of Eukaryotic Microbiology* **50** (Suppl.), 682–684.

Hamour A, Curry A, Ridge A, Baily G, Wilson G and Mandal B (1997) *Isospora belli* in a patient with AIDS. *Journal of Infection* **35**, 94–95.

Henderson HE, Gillepsie GW, Kaplan P and Steber M (1963) The human *Isospora*. *American Journal of Tropical Medicine and Hygiene* **78**, 302–309.

Henry K, Bird RG and Doe WF (1974) Intestinal coccidiosis in a patient with alpha-chain disease. *British Medical Journal* **1**, 542–543.

Jarpa Gana A (1966) Coccidiosis humana. *Biologica (Santiago)* **39**, 3–26.

Jeffery GM (1956) Human coccidiosis in South Carolina. *Journal of Parasitology* **42**, 491–495.

Jongwutiwes S, Sampatanukul P and Putaporntip C (2002) Recurrent isosporiasis over a decade in an immunocompetent host successfully treated with pyrimethamine. *Scandinavian Journal Infectious Diseases* **34**, 859–862.

Kim MJ, Kim WH, Jung HC, Chai JW and Chai JY (2013) *Isospora belli* infection with chronic diarrhea in an alcoholic patient. *Korean Journal of Parasitology* **51**, 207–212.

Lagrange-Xélot M, Porcher R, Sarfati C, de Castro N, Carel O, Magnier JD, Delcey V and Molina JM (2008) Isosporiasis in patients with HIV infection in the highly active antiretroviral therapy era in France. *HIV Medicine* **9**, 126–130.

Lai KK, Goyne HE, Hernandez-Gonzalo D, Miller KA, Tuohy M, Procop GW, Lamps LW and Patil DT (2016) *Cystoisospora belli* infection of the gallbladder in immunocompetent patients: a clinicopathologic review of 18 cases. *American Journal of Surgical Pathology* **40**, 1070–1074.

Legua P and Seas C (2013) *Cystoisospora* and *Cyclospora*. *Current Opinion in Infectious Diseases* **26**, 479–483.

Levine ND (1973) *Protozoan Parasites of Domestic Animals and of Man*, 2nd edn. Minneapolis, Minnesota, USA: Burgess, pp. 1–406.

Liebman WM, Thaler MM, DeLorimier A, Brandborg LL and Goodman J (1980) Intractable diarrhea of infancy due to intestinal coccidiosis. *Gastroenterology* **78**, 579–584.

Lindsay DS, Dubey JP, Toivio-Kinnucan MA, Michiels JF and Blagburn BL (1997) Examination of extraintestinal tissue cysts of *Isospora belli*. *Journal of Parasitology* **83**, 620–625.

Martelli MG and Lee JY (2016) Parasitic infection of the gallbladder: *Cystoisospora belli* infection as a cause of chronic abdominal pain and acalculous cholecystitis. *Journal of the Mississippi State Medical Association* **57**, 174–176.

Matsubayashi H and Nozawa T (1948) Experimental infection of *Isospora hominis* in man. *American Journal of Tropical Medicine Hygiene* **28**, 633–637.

Meamar AR, Rezaian M, Zahabium F, Faghihi AH, Oormazdi H and Kia EB (2009) Severe diarrhea due to *Isospora belli* in a patient with thymoma. *Journal of Microbiology, Immunology and Infection* **42**, 526–529.

Michiels JF, Hofman P, Bernard E, Saint Paul MC, Boissy C, Mondain V, Lefichoux Y and Loubiere R (1994) Intestinal and extraintestinal *Isospora belli* infection in an Aids patient. A second case report. *Pathology Research and Practice* **190**, 1089–1093.

Modigliani R, Bories C, LeCharpentier Y, Salmeron M, Messing B, Galian A, Rambaud JC, Lavergne A, Cochand-Priollet B and Desportes I (1985) Diarrhoea and malabsorption in Acquired Immune Deficiency Syndrome: a study of four cases with special emphasis on opportunistic protozoan infestations. *Gut* **26**, 179–187.

Murphy SC, Hoogestraat DR, Sengupta DJ, Prentice J, Chakrapani A and Cookson BT (2011) Molecular diagnosis of cystoisosporiasis using extended-range PCR screening. *Journal of Molecular Diagnostics* **13**, 359–362.

Niedmann G (1963) Elementos parasitarios en la mucosa del intestino delgado en biopsias de enfermos con isosporosis. *Boletín Chileno de Parasitología* **18**, 22.

Ortega YR, Nagle R, Gilman RH, Watanabe J, Miyagui J, Quispe H, Kanagusuku P, Roxas C and Sterling CR (1997) Pathologic and clinical findings in patients with cyclosporiasis and a description of intracellular parasite life-cycle stages. *Journal of Infectious Diseases* **176**, 1584–1589.

Peng CY and Tsai W (1991) *Isospora belli* infection in a patient with Hodgkin's disease: report of a case. *Journal of the Formosan Medical Association* **90**, 260–263.

Rao ACK, Geetha V, Ranjini K, Vidhyalakshmi S and Rupashree S (2012) Histology as a diagnostic tool for intestinal isosporiasis in immunocompromised patients. *Asian Pacific Journal of Tropical Disease* **2012**, 251–252.

Restrepo C, Macher AM and Radany EH (1987) Disseminated extraintestinal isosporiasis in a patient with acquired immune deficiency syndrome. *American Journal of Clinical Pathology* **87**, 536–542.

Sasaki M, Tanaka A, Nishimura T, Tsujikawa T, Andoh A, Ishizuka I, Koyama S, Fujiyama Y and Kushima R (2004) A case of malabsorption syndrome caused by isosporiasis in an immunocompetent patient. *Journal of Gastroenterology* **39**, 88–89.

Swanson EA, March JK, Clayton F, Couturier MR, Arcega R, Smith R and Evason KJ (2018) Epithelial inclusions in gallbladder specimens mimic parasite infection: histologic and molecular examination of reported *Cystoisospora belli* infection in gallbladders of immunocompetent patients. *American Journal of Surgical Pathology* **42**, 1346–1352.

https://doi.org/10.1017/S003118201900012X Published online by Cambridge University Press

Tatum A (1916) The influence of bile on autolysis. *Journal of Biochemistry* **27**, 243–248.

Trier JS, Moxey PC, Schimmel EM and Robles E (1974) Chronic intestinal coccidiosis in man: intestinal morphology and response to treatment. *Gastroenterology* **66**, 923–935.

Velásquez JN, Carnevale S, Mariano M, Kuo LH, Caballero A, Chertcoff A, Ibáñez C and Bozzini JP (2001) Isosporosis and unizoite tissue cysts in patients with acquired immunodeficiency syndrome. *Human Pathology* **32**, 500–505.

Velásquez JN, Osvaldo GA, Di Risio C, Etchart C, Chertcoff AV, Perissé GE and Carnevale S (2011) Molecular characterization of *Cystoisospora belli* and unizoite tissue cyst in patients with acquired immunodeficiency syndrome. *Parasitology* **138**, 279–286.

Velásquez JN, di Risio CA, Etchart CB, Chertcoff AV, Nigro MG, Pantano ML, Ledesma BA, Vittar N and Carnevale S (2016) First report of *Cystoisospora belli* parasitemia in a patient with acquired immunodeficiency syndrome. *Acta Parasitologica* **61**, 172–177.

Veldhuyzen van Zanten SJO, Lange JMA, Sauerwein HP, Rijpstra AC, Laarman JJ, Rietra PJGM and Danner SA (1984) Amprolium for coccidiosis in AIDS. *Lancet* **2**, 345–346.

Walther Z and Topazian MD (2009) *Isospora* cholangiopathy: case study with histologic characterization and molecular confirmation. *Human Pathology* **40**, 1342–1346.

Wenyon CM (1923) Coccidiosis of cats and dogs and the status of the *Isospora* of man. *Annals of Tropical Medicine Parasitology* **17**, 231–288.

https://doi.org/10.1017/S003118201900012X Published online by Cambridge University Press