


Unexpectedly High Prevalence of *Cystoisospora belli* Infection in Acalculous Gallbladders of Immunocompetent Patients

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Key Words: *Cystoisospora belli*; Gallbladder; Cholecystectomy

Am J Clin Pathol January 2019;151:100-107

DOI: 10.1093/AJCP/AQY122

ABSTRACT

Objectives: Indications for cholecystectomy have changed dramatically over the past three decades. *Cystoisospora belli* has been reported in cholecystectomy specimens of immunocompetent patients. The present study was designed to determine the prevalence and clinical association of *C belli* in the gallbladder.

Methods: The study included retrospective review of cholecystectomy specimens ($n = 401$) removed for various indications, and a prospective cohort of cholecystectomy specimens ($n = 22$) entirely submitted for histologic evaluation. Correlations of presence of *C belli* with age, sex, clinical indication, and abnormalities of preoperative laboratory values were assessed by Fisher exact test.

Results: *C belli* was identified in 39/401 (9.7%) of the retrospective cohort, and 6/22 (27.3%) of the entirely submitted specimens. The presence of *C belli* showed no correlation with age, sex, clinical indication, or laboratory abnormalities.

Conclusions: *C belli* resides in a latent state in the gallbladder and may be best considered a commensal organism.

Biliary pain in the absence of gallstones or sludge presents a complicated and difficult clinical problem, often termed functional gallbladder disorder (FGBD), chronic cholecystitis, acalculous biliary pain, biliary dyskinesia, gallbladder dysmotility, or sphincter of Oddi dysfunction.¹ The diagnosis, conceptual classification, and management of FGBD are controversial.

A recent review of the New York State Planning and Research Cooperative System Longitudinal Administrative Database (spanning 1995-2013) revealed that the indications for cholecystectomy have changed dramatically in the past 20 years. By comparison of data from 2010 to that of 1997, calculous cholecystitis declined (-20%; $P < .0001$), while other indications increased, including acalculous cholecystitis (+94%; $P < .0001$), biliary dyskinesia (+331%; $P < .0001$), and biliary colic (+55%; $P = .0013$).² There has been a concomitant decrease in the age of patients undergoing cholecystectomy,³ and a shift from open cholecystectomy to laparoscopic cholecystectomy as standard of care. While similar trends have been reported in a survey of the Nationwide Inpatient Sample database, these trends have not been reported outside of the United States, as evidenced by examinations of patient databases in Sweden, Norway, Australia, and Poland.⁴

While laparoscopic cholecystectomy is associated with shorter postoperative stay and fewer minor complications (eg, wound infections),⁵ there is a five-fold increase

in the incidence of the devastating long-term complication of bile duct injury among patients who undergo laparoscopic cholecystectomy compared to open cholecystectomy.^{6,7} The sequelae of bile duct injury include serious morbidity,⁸ reduced quality of life,⁹ and reduced long-term survival,¹⁰ resulting in litigation costs estimated at \$1 billion annually in the United States.¹¹ Thus, understanding the etiology of gallbladder disease, particularly in the setting of FGBD, is important from both clinical and pathobiological standpoints.

Cystoisospora are coccidian protozoan parasites of the phylum Apicomplexa. *C belli* and *C natalensis* are the only *Cystoisospora* species known to infect humans, with *C belli* representing the vast majority of reported cases.¹² *C belli* is a poorly understood organism; humans represent the only known host of this parasite and no known reservoir or paratenic hosts exist.¹³ Symptomatic infections typically result in secretory diarrhea¹⁴ and are diagnosed by microscopic examination of small intestinal biopsies for organisms or, more commonly, stool specimens examined for oocysts. *C belli* initially came to prominent medical attention as an opportunistic infection complicating acquired immunodeficiency syndrome (AIDS), where infection of the small intestine epithelium resulted in severe, secretory diarrhea¹⁵ and rarely disseminated extraintestinal infection.^{16,17} There are also a few reports of HIV-positive patients with chronic cholecystitis who were found to have *C belli* infection in the resected gallbladder specimen.^{18,19} Another case report demonstrated biopsy-proven symptomatic *C belli* infection of the common bile duct in a patient who presented with common bile duct obstruction, and who was concurrently diagnosed with infections by *Strongyloides stercoralis* and HIV.²⁰ The reported prevalence of *C belli* in patients with AIDS varies widely, likely due to differences in geographic location and patient populations. Subsequent case reports have documented instances of *C belli* diarrhea in the setting of other causes of profound immunodeficiency, such as solid organ transplant,²¹ thymoma,²² inflammatory bowel disease/azathioprine exposure,²³ malnutrition,²⁴ and alcohol abuse.²⁵ These studies resulted in the general consensus that *C belli* is a rare opportunistic infection seen in immunocompromised patients that results in diffuse watery diarrhea, with greater prevalence in tropical climates and in patients with recent travel history.^{26,27}

There have also been case reports of self-limited *C belli* infection in immunocompetent patients,²⁸ as well as several larger case series suggesting that conventional stool studies performed to detect coccidian oocysts may have low sensitivity,²⁹ raising the possibility that *C belli* is more prevalent amongst immunocompetent humans than previously recognized. A recent case report of

C belli infection in the gallbladder of an immunocompetent patient³⁰ was followed by a retrospective case series describing the clinicopathologic features of 18 cholecystectomies with diagnosed *C belli* infection.³¹ This case series found that the majority of gallbladders with reported *C belli* infection represented acalculous disease in younger patients, but given the limitations of the study could not assess prevalence of *C belli*. The current study investigates the prevalence of *C belli* in resected human gallbladders in a larger cohort, and whether its presence is associated with the indication for cholecystectomy and laboratory data.

Materials and Methods

We designed a single-institution, two-arm study to determine the prevalence of *C belli* infection in the human gallbladder. With institutional research board approval, the laboratory information system of the surgical pathology division at the University of Rochester Medical Center was searched for cholecystectomies, excluding the terms cholelith or calculous (2004-2016). Four hundred and one patients with confirmed absence of gallstones on pathologic review were included in the retrospective review. The original H&E slides were reviewed and the presence or absence of *C belli* organisms was correlated with age, sex, and the clinical indication for cholecystectomy for all 401 patients, and correlated with available clinical (age, sex, presenting complaint, indication for the procedure, comorbidities, immune status, medication list, and body mass index [BMI]) and laboratory values, including presence/absence of leukocytosis at the time of procedure, peripheral eosinophilia, alkaline phosphatase, bilirubin, medications, and radiologic data (hepatobiliary iminodiacetic acid [HIDA] scan results) for 235 of these patients.

The indication for cholecystectomy was classified into six categories: cholecystitis/abdominal pain, gallstone disease (cholelithiasis/choledocholithiasis, gallstone pancreatitis), biliary dyskinesia, gallbladder polyp, choledochal cyst, and incidental (gallbladders removed for reasons other than symptoms of gallbladder disease). SPSS version 25.0 (IBM, Armonk, NY) was used to query the data for associations between patient demographics and clinical features, with the presence of *C belli* via Fisher exact test, with a *P* value less than .05 selected as a threshold for statistical significance.

Because routine sampling of cholecystectomy specimens typically involves histologic evaluation of less than 5% of the specimen, there is a significant potential for underestimating the prevalence of *C belli*. Given that the

previous case series found the majority of patients diagnosed with *C belli* of the gallbladder were young women with acalculous disease,³¹ we designed a separate, parallel, prospective study (July 2017-February 2018), in which gallbladders from any patient less than 30 years of age that lacked stones and lacked organisms on the initial routine slide were entirely submitted for histologic evaluation (n = 22).

In both arms of the study, all cases that were positive for *C belli* were evaluated by at least two GI pathologists, with agreement. Historically, morphologic speciation of *Cystoisospora* has been based on microscopic examination of oocysts in stool, and thus the lack of stool ova and parasite examinations from these patients precludes definite identification of species. However, human infection by *C natalensis* has only been reported in a single case report (1953) of a 21-year-old man with concurrent amoebic dysentery and other protozoal and helminthic infections and has not been reported in humans since.¹² Therefore, we feel it is reasonable to refer to the organism discussed here as *C belli*.

Results

The retrospective cohort of patients who underwent cholecystectomy without gallstones (n = 401) included 265 (66.1%) females and 136 (33.9%) males. The mean age was 33.1 years (\pm 20.4 years). The population of patients undergoing cholecystectomy showed a bimodal age distribution, with a peak at 13 to 25 years of age and another at 50 to 70 years (Figure 1). The latter group had a higher proportion of males, as well as an increased proportion of incidental cholecystectomies performed in the setting of liver transplantation and resections for malignancy (Table 1). Indications for removal were biliary dyskinesia (124/401; 30.9%), abdominal pain/cholecystitis (103/401; 25.7%), gallstone disease (78/401; 19.5%), gallbladder polyp (17/401; 4.2%), choledochal cyst (4/401; 1.0%), and incidental (75/401; 18.7%). Incidental cholecystectomies included gallbladders removed in the setting of trauma (n = 21), liver explant (n = 21), pancreatic/duodenal neoplasm (n = 16), pancreatitis (n = 6), liver mass (n = 7), and other abdominal surgeries (n = 4). The distribution of indication by patient age is also shown in Figure 1. The prevalence of *C belli* as it relates to age is shown in Figure 1, and the prevalence of *C belli* as it relates to clinical setting is shown in Table 1.

Of the entire retrospective cohort, *C belli* was identified in 39/401 (9.7%) specimens. *C belli* was identified in 15/136 (11.0%) of males and 24/265 (9.1%) of females ($P = .594$); 26/240 (10.8%) of positive cases were from

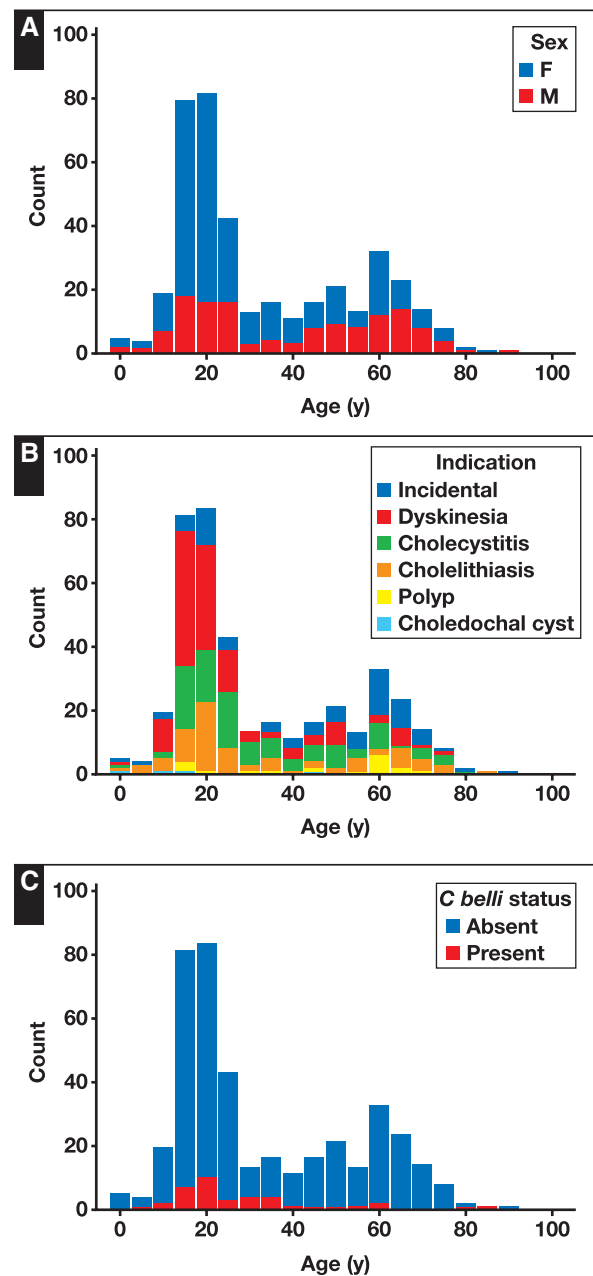


Figure 1 Distribution of patient sex (A), indication for cholecystectomy (B), and *Cystoisospora belli* infection (C) by age (retrospective cohort; n = 401).

patients younger than 30 years, and 13/161 (8.1%) of positive cases were from patients 30 years or older ($P = .395$). No significant association with indication for cholecystectomy was identified ($P = .743$). Two of the 401 patients (both negative for *C belli* in gallbladder) had stool studies for ova/parasites performed during workup for diarrhea, neither of which were positive. Of the 39 positive patients, 11 had previous duodenal biopsies during their workup, none of which showed definitive *C belli* infection. In the 235 patients with available clinical and laboratory data

Table 1
Patient Demographics of the Entire Cohort Of Patients Included in the Retrospective Review

Characteristic	Total, No. (%) (n = 401)	<i>C belli</i> Present, No. (%) (n = 39)	<i>C belli</i> Absent, No. (%) (n = 362)	<i>P</i> ^a
Age <40	240 (59.9)	26 (66.7)	214 (59.1)	.075
Male	136 (33.9)	15 (38.5)	121 (33.4)	.594
Indication				
Dyskinesia	124 (30.9)	12 (30.1)	112 (30.9)	.743
Cholecystitis	103 (25.7)	8 (20.5)	95 (26.2)	
Gallstone disease	78 (19.5)	7 (17.9)	71 (19.6)	
Polyp	17 (4.3)	1 (2.6)	16 (4.4)	
Choledochal cyst	4(1.0)	0	4 (1.1)	
Incidental	75 (18.7)	11 (28.2)	64 (17.7)	

C belli, *Cystoisospora belli*.

^aFisher exact test, two-sided.

Table 2, the presence of *C belli* was associated with male gender but not with elevated BMI, leukocytosis, peripheral eosinophilia, elevated alkaline phosphatase, elevated total bilirubin, proton pump inhibitor use, or antibiotic exposure (within 2 weeks prior to surgery).

From the cohort of patients in the prospective arm of the study, in which gallbladders were entirely submitted for histologic evaluation, *C belli* was noted in six of 22 (27.3%). The mean routine slides submitted were 1.14 slides/case; the mean total slides after entire submission of the specimen were 13.3 slides/case. In three cases, *C belli* was identified on the initial (routine) slide; in three additional cases *C belli* was identified on subsequent slides that would not have been sampled by routine processing. *C belli* was identified in the cystic duct margin in one of six (16.7%) and only in the remaining gallbladder sections in five of six (83.3%) positive cases. Of the 22 patients, cholecystectomy was performed for the following indications: eight (36.4%) biliary dyskinesia, six (27.2%) cholelithiasis, five (22.7%) chronic cholecystitis/pain, two (9.1%) for gallbladder polyps, and one (4.5%) was removed incidentally (partial hepatectomy for metastatic colorectal carcinoma) **Table 3**. The indications for removal of specimens positive for *C belli* included biliary dyskinesia (three), cholelithiasis (one), chronic cholecystitis (one), and gallbladder polyp (one). One of the six patients with *C belli* infection of the gallbladder had duodenal biopsies performed prior to cholecystectomy; on review, *C belli* organisms were not identified. One patient diagnosed with *C belli* infection received 2 weeks of antibiotics (trimethoprim/sulfamethoxazole × 1 week, then ciprofloxacin × 1 week) after surgery, and re-presented 4 months later with abdominal discomfort. Stool studies with trichrome stain performed at that time were negative.

Table 2
Clinical and Laboratory Data for 235 Patients Included in the Retrospective Review

Characteristic	Total, No. (%) (n = 235)	<i>C belli</i> Present, No. (%) (n = 24)	<i>C belli</i> Absent, No. (%) (n = 211)	<i>P</i> ^a
Male	62 (26.4)	11 (45.8)	51 (24.2)	.029
Overweight	111 (47.2)	7 (29.2)	104 (49.3)	.13
Leukocytosis	34 (14.5)	8 (33.3)	26 (12.3)	.063
Eosinophilia	11 (4.7)	2 (8.3)	9 (4.3)	.211
Alkaline phosphatase	16 (6.8)	2 (8.3)	14 (6.6)	.827
Total bilirubin	27 (11.5)	2 (8.3)	25 (11.8)	.859
PPI	63 (26.8)	6 (25.0)	57 (27.0)	.612
Antibiotic	35 (14.9)	5 (20.8)	30 (14.2)	.551

C belli, *Cystoisospora belli*; PPI, proton pump inhibitor.

^aFisher exact test.

Table 3
Clinical Indication for the Prospective Cohort

Indication	Total, No. (%) (n = 22)	<i>C belli</i> Present, No. (%) (n = 6)	<i>C belli</i> Absent, No. (%) (n = 16)	<i>P</i> ^a
Male	4 (18.2)	0	4 (25)	
Indication				
Dyskinesia	8 (36.4)	3 (50)	5 (31.3)	.831
Cholecystitis	5 (22.7)	1 (16.7)	4 (25)	
Gallstone disease	6 (27.2)	1 (16.7)	5 (31.3)	
Polyp	2 (9.1)	1 (16.7)	1 (6.3)	
Incidental	1 (4.5)	0	1 (6.3)	

C belli, *Cystoisospora belli*.

^aFisher exact test.

The histology of *C belli* in the gallbladder is distinctive on H&E staining, featuring a prominent intraepithelial parasitophorous vacuole within the surface epithelium, most often in the absence of inflammation **Image 1**. In the majority of cases, the parasite burden consisted of scattered inconspicuous clusters of inhabited epithelium; less commonly, there was patchy or moderately diffuse involvement of the epithelium. One potential histologic mimic, hyaline globules (also known as thanatosomes),³² can be distinguished by the presence of epithelial injury with attendant inflammation, lack of a distinct vacuole, and consistent negative staining by Alcian blue (**Image 1**).³²

Discussion

This study reports the largest sample, to our knowledge, of gallbladders that have been systematically examined for *C belli*. The principal finding is that this organism is more prevalent among immunocompetent humans than previously recognized. The prevalence of *C belli* in stool specimens has been evaluated in several different settings.

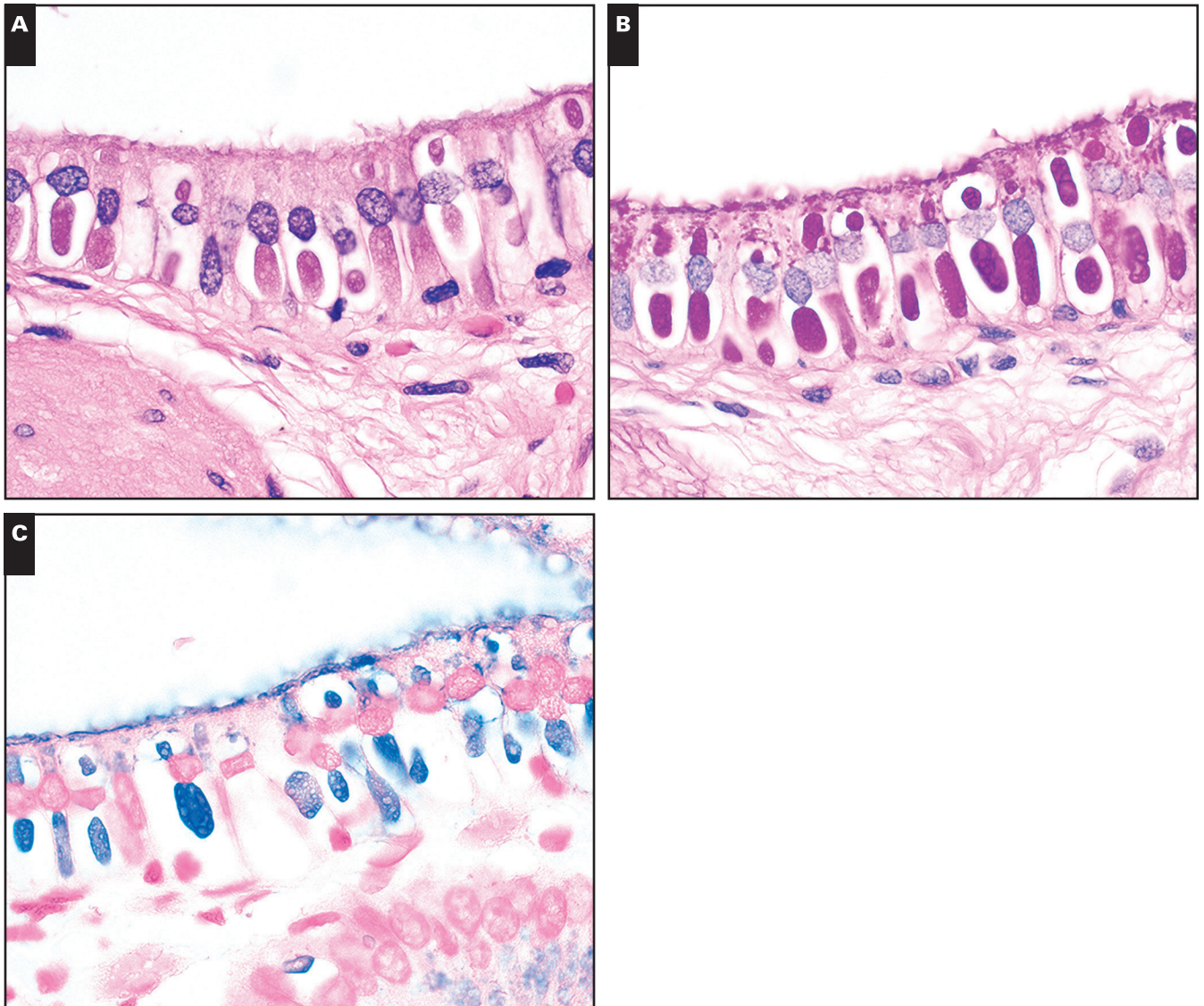


Image 1 *Cystoisospora belli* is characterized by a distinctive parasitophorous vacuole (**A**, H&E, $\times 1,000$), and is highlighted by periodic acid-Schiff-diastrase (PASD) (**B**, $\times 1,000$) and Alcian blue histochemical stains (**C**, $\times 1,000$).

A review of the ledgers of King George Hospital found that 2.6% of 384 patients showed scant *C belli* oocysts in stool³³; nearly all these patients were soldiers (presumably immunocompetent) returning from Egypt and Gallipoli during World War I. In the authors' opinion, "no ill effect" was attributed to the presence of *C belli* oocysts in these patients' stool. Perhaps the best prevalence data reported to date, which have varied widely, are from several large cohort studies of patients with diarrhea in the setting of AIDS, in which symptomatic *C belli* infection was diagnosed via microscopic examination of stool samples for *C belli* oocysts. A study of 16,351 patients with HIV/AIDS in Los Angeles, CA (spanning 1985-1992), found an overall prevalence of 1.0%, with highest prevalence among patients from El Salvador (7.4%) and Mexico (5.4%).²⁶ Another

study, which utilized the French Hospital Database on HIV, reported 17 cases among 26,815 enrolled, with *C belli* infection greatest among patients from sub-Saharan Africa.³⁴ That same study compared their findings to a later time period in which combination antiretroviral medications were in use, and found that combination antiretroviral therapy reduced persistent *C belli* infection on follow-up by 78% (with no difference between the two time periods among patients with CD4 T-cell counts $< 50/\mu\text{L}$). This confirmed that persistent symptomatic infection occurs predominantly in the setting of pronounced immunodeficiency. An interesting study from India included stool studies from patients of unknown immune status who presented with diarrhea ($n = 200$), and control patients without diarrhea ($n = 50$), finding a prevalence of 22% and 4% in those with and

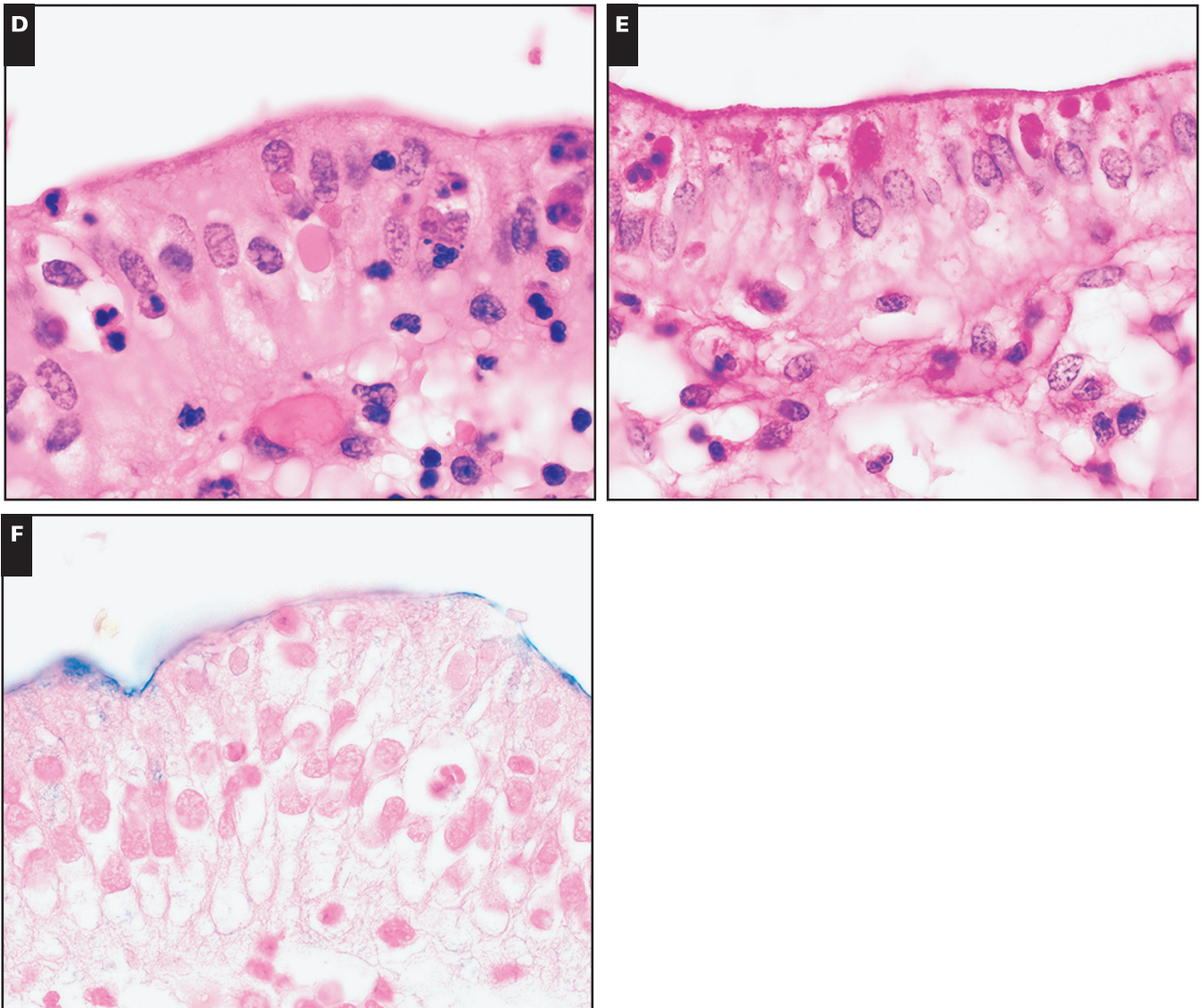


Image 1 (cont) Hyaline globules represent one histologic mimic (**D**, H&E, $\times 1,000$) and also stain positive with PASD (**E**, $\times 1,000$), but are consistently negative for Alcian blue (**F**, $\times 1,000$).

without diarrhea, respectively, and indicating that, at least in some populations, asymptomatic infection is not uncommon in humans. The variability of prevalence data has been interpreted to reflect differences in patient demographics, with patients from endemic areas having higher rates. Data regarding the prevalence of oocysts in surface water are scarce; one report of potable drinking water from Dakahlia, Egypt identified *C belli* oocysts in 0.47% of 840 samples,³⁵ suggesting that, at least in some regions, exposure to *C belli* oocysts may be more common than previously realized.

Our cohort of healthy immunocompetent patients without diarrhea revealed a prevalence of *C belli* in gallbladder resections that was similar to the prevalence of symptomatic infections in patients with AIDS and diarrhea diagnosed by microscopic examination of stool

studies, and greater than the reported prevalence of *C belli* oocysts in the stool of patients without diarrhea in India. While at first glance these findings may seem unexpected, we believe they make sense in the setting of several observations.

Cryptosporidium parvum is a similar parasite that has caused large outbreaks in the United States when drinking water or recreational water becomes contaminated by bovine feces.³⁶ *C parvum* oocysts are infectious upon excretion in the feces, and very few oocysts are required to initiate symptomatic infection. In contrast, the *Cystoisospora* oocysts are not infectious at the time of excretion, requiring maturation outside of the body, a process that requires 1 to 2 weeks.¹¹ This presumably decreases the efficiency of infection and the density of infectious oocysts during

a typical exposure. Furthermore, humans are the only known host of *C belli*, making large-scale contamination of drinking water less frequent. Given the low infectious rate and the lack of a nonhuman reservoir, it would seem that the symptomatic infections of (typically immunocompromised) patients would require a population of asymptomatic human carriers that shed oocysts at a (presumably) low rate. Because *Cystoisospora* species hatch when exposed to bile,³⁷ yet are not commonly appreciated in duodenal biopsies that are examined carefully for subtle abnormalities in routine diagnostic practice, it seems reasonable that *C belli* may reside in a relatively latent state with low organismal burden in the gallbladder, having been overlooked by surgical pathologists in an organ where clinical management does not typically rely on thorough tissue sampling or high magnification microscopic examination.

The presence of *C belli* in cholecystectomy specimens has several important differences from symptomatic infections of the biliary tree and small intestine. First, symptomatic infection of the small intestine is invariably associated with secretory diarrhea, often severe enough to require hospitalization. Second, the histology of symptomatic infection by *C belli* shows numerous organisms accompanied by active inflammation. Stool studies are positive for oocysts. In our cohort of gallbladder resections from immunocompetent patients, the presence of *C belli* is characterized by a lack of diarrhea, a lack of inflammatory response, and a lack of an association with any of the clinical settings that led to cholecystectomy. While the presence of *C belli* in human gallbladders does not explain the changing demographics of cholecystectomy, the unexpectedly high prevalence in gallbladder specimens indicates that *C belli* frequently inhabits humans and may instead be better conceived as a commensal organism with a relatively latent presence in the gallbladder, only giving rise to symptomatic infection in the setting of pronounced immunodeficiency and/or immunosuppression.

There are several limitations to this study. The retrospectively studied population here was biased toward younger patients and excluded cases with cholelithiasis seen on gross examination. However, even with the relative undersampling of patients over 30 years of age, *C belli* was identified in patients up to 87 years of age, and even this cohort of grossly acalculous gallbladders included 78 gallbladders removed for gallstone disease. A second limitation is the lack of a second, confirmatory method (ie, polymerase chain reaction and/or electron microscopy). However, all of our cases were confirmed on review by at least two GI pathologists, and because the distinctive H&E appearance is sufficient for diagnostic practice to guide clinical decision making, we feel the added expense of these studies is unnecessary.

Many facets of the biology of this fascinating organism remain unknown. For instance, does *C belli* infection cause symptoms when colonization is initially established? Do protozoal infections explain a subset of self-limited diarrheal episodes currently presumed to represent viral gastroenteritis? If the presence of *C belli* in the gallbladder represents latent infection as we propose, how frequently do these patients shed oocysts? Do paratenic hosts (domesticated cats, dogs, etc) play an unrecognized role in transmission? Further studies of this poorly understood organism are needed.

In conclusion, our data show that *C belli* frequently inhabits the human gallbladder at a prevalence of at least 9.7% (likely closer to 27%), but the presence of *C belli* shows no association with the clinical indication for cholecystectomy, and indeed is not increased in the setting of cholecystectomy performed for gallbladder-related symptoms. As such, we would not advise submitting additional sections for the purpose of increasing likelihood of *C belli* detection for routine patient care. Given the heterogeneous clinical context and the morphologic findings reported here, we propose that the presence of *C belli* in the human gallbladder represents a latent anatomic reservoir, and may be best classified as a commensal organism in the setting of an immunocompetent patient.

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*H.D.N. and K.S. are undergraduate students from other institutions who performed their contribution to the project while participating in the PathIT program at University of Rochester Medical Center.

This work was supported by the University of Rochester Medical Center Department of Pathology and Laboratory Medicine Faculty Research Fund.

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