The role of gastrointestinal pathogens in inflammatory bowel disease: a systematic review

Jordan E. Axelrad, Ken H. Cadwell, Jean-Frederic Colombel and Shailja C. Shah

Abstract: The inflammatory bowel diseases (IBD), comprising Crohn’s disease (CD) and ulcerative colitis (UC), are chronic, progressive, inflammatory conditions of the gastrointestinal tract. Imbalance in the gut microbial community, or dysbiosis, and the subsequent immune response, represent the critical relationship between genetic susceptibility, microbes, and environment factors, that result in IBD. Gastrointestinal pathogens – a common cause of dysbiosis – have been implicated as an environmental trigger in new onset IBD, as well as flare of existing IBD. In this article, we systematically review clinical data regarding the association between specific gastrointestinal pathogens and IBD. Numerous bacteria, viruses, fungi, and parasites have been implicated in the pathogenesis of IBD, and exacerbations of existing disease. In this article, we will also specifically discuss the less recognized microbes that have an inverse association with IBD, including certain bacterial pathogens, such as Helicobacter pylori, and parasites, such as Trichuris species. Future prospective and experimental studies are required to establish causality and clarify potential mechanisms of enteric pathogens in modifying the risk and course of IBD.

Keywords: Enteric infection, gastroenteritis, inflammatory bowel disease, pathogenesis, microbiome, flare, mucosal immunology
Enteric infections, leading culprits in gut microbial dysbiosis, are notoriously implicated as environmental triggers in new onset IBD or flare of existing IBD.10–14 However, enteric infections are not all the same. Indeed, some enteric infections are of no consequence, and perhaps even less recognized is the potential benefit of some gastrointestinal microbes, such as Helicobacter pylori and intestinal helminths, which have been associated inversely with risk of IBD. The ever-increasing health, societal, and economic burden of IBD worldwide highlights the urgent need to identify modifiable risk and protective factors implicated in IBD pathogenesis. We recently reviewed translational data implicating gastrointestinal pathogens in incident IBD.15 Thus, our review aimed to identify modifiable risk and protective factors of IBD worldwide highlighting the urgent need to identify modifiable risk and protective factors implicated in IBD pathogenesis. We recently reviewed translational data implicating gastrointestinal pathogens in incident IBD.15 Thus, our primary objective was to conduct a comprehensive, clinical systematic review to define the association between specific gastrointestinal infections and (1) new onset IBD and (2) disease relapse, as well as provide an appraisal of specific studies to help with interpretation of current as well as future literature.

Methods

Search strategy

We searched electronic medical databases: PUBMED, Ovid, Scopus, ScienceDirect, CINAHL, LILACS, IMBIOMED, Scielo, IngentaConnect, Nature Publishing Group, and Cochrane database, from January 1990 to January 2020 for all English human and non-human studies assessing IBD and gastrointestinal infections. MeSH terms and/or text words included the broad terms inflammatory bowel disease, gastrointestinal infection, enteric infection. We also searched specific pathogens including bacteria (Campylobacter species, Helicobacter species, Mycobacterium avium paratuberculosis, Clostridioides difficile, Listeria monocytogenes, Yersinia enterocolitica, Salmonella species, Escherichia coli, Vibrio species, Plesiomonas shigeloides), viruses (Adenovirus, Astrovirus, Norovirus, Rotavirus, Sapovirus, cytomegalovirus, Epstein–Barr virus, human herpes virus 6), parasites (Trichuris species, Cryptosporidium species, Cyclospora cayetanensis, Entamoeba histolytica, Giardia lamblia), and fungi (Candida species, Aspergillus species, Cryptococcus neoformans), combined with “AND inflammatory bowel disease OR Crohn disease OR colitis, ulcerative OR indeterminate colitis.” We additionally reviewed the references of pertinent review articles and the studies meeting inclusion criteria for additional relevant articles.

Study selection

Experimental data, including specific gut microbiome and molecular changes associated with enteric infections in the absence of a diagnosis of new onset IBD or clinical disease flare is outside of the scope of this review. Clinical trials, cohort studies, and cross-sectional studies were eligible for inclusion. Case-series, review articles, and conference abstracts were excluded. Studies were included if they met the following inclusion criteria: (1) patients diagnosed with IBD [CD, UC, or IBD-undifferentiated (IBD-U)] according to standard diagnostic criteria; (2) diagnosis of gastrointestinal infection as defined by positive testing on any of the following modalities: histology, culture, serology, polymerase chain reaction (PCR) and/or other molecular technique as long as defined in the study; (3) documentation of criteria for disease flare qualification (e.g., clinical criteria, endoscopic criteria); (4) sufficient information provided to interpret or calculate comparative effect estimates; and (5) full-text available in English. We additionally documented the time course of gastrointestinal infection relative to incident IBD or disease flare, where available, such as in cohort studies. Of the 2002 unique studies identified, 418 studies were reviewed in full text, of which 97 met inclusion criteria for clinical studies of pathogens and IBD (Figure 1, Supplemental Table S1).

Gastrointestinal pathogens and increased risk of IBD

Bacteria

Many observational, cross-sectional or retrospective case-control and cohort studies link specific bacterial pathogens as potential triggers for new onset clinical IBD. Although no singular, causative microorganism or associated time course has been identified, several association studies implicate Campylobacter species, Salmonella species, enterohemorrhagic Helicobacter species (EHS), Mycobacterium avium paratuberculosis (MAP), Clostridioides difficile, and Listeria monocytogenes (Table 1).

Campylobacter species. In a nationwide cohort study from Denmark of 49,420 stool cultures positive for Campylobacter jejuni with 94,264,447
patient-years of follow up, both UC and CD were more common following an episode of gastroenteritis where the stool culture was positive for *Campylobacter* [UC IRR (incidence rate ratio) 2.6, 95% confidence interval (CI) 2.3–3.0; CD IRR 2.2, 95% CI 1.8–2.7] compared with patients without gastroenteritis. However, the incidence of UC and CD was significantly higher after a negative compared with a positive stool culture (UC IRR 8.2, 95% CI 8.0–8.5; CD IRR 6.4, 95% CI 6.1–6.7), suggesting possible detection bias.16 Despite the large sample size and duration of follow up, there may be significant selection or indication bias. Antimicrobial therapies administered contemporaneously were also not considered by the study investigators.16,17 Based on a more recent nationwide case-control study from Sweden of 480,721 patients, a diagnosis of *Campylobacter* species was associated with higher odds of UC [adjusted odds ratio (aOR), 1.86; 95% CI 1.32–2.61] and CD (aOR, 95% CI 1.87; 1.13–3.11) after adjusting for several factors including age, sex, birth year, place of residence, previous gastrointestinal surgery, autoimmune disease, and family history of IBD.18

Studies often analyze subset species as a composite genus group. This is particularly relevant for *Campylobacter* species, many of which are recognized as human pathogens and implicated in IBD pathogenesis, namely, *Campylobacter jejuni*, *C. coli*, *C. urolyticus*, *C. showae*, and *C. concisus*. In particular, *C. concisus*, which has a virulence-associated restriction-modification system, has diverse pathogenic functions, including the ability to adhere to and invade host cells and secrete toxins.19 In a study of pediatric patients, *C. concisus* antibodies and DNA were detected with significantly higher frequency in patients with CD compared with patients without IBD.20,21 Among adults, one study demonstrated that *C. concisus* DNA was detected with significantly higher frequency in colonic biopsies of patients with CD (53%) and UC (77%) compared with adults without CD or UC (18% and 36%, respectively). These data have been confirmed in several other
Given the substantial genetic and functional diversity among \textit{C. concisus} strains, further research is required.

There are some inconsistencies, as genus-specific PCR analyses have not demonstrated a significant difference between the prevalence of \textit{Campylobacter} species in intestinal biopsies of patients with IBD versus those without IBD.\textsuperscript{26} In addition, in a broad meta-analysis comprising nine studies including 519 patients with IBD and 1133 non-IBD controls, \textit{C. concisus} [pooled odds ratio (pOR) 3.76, 95\% CI 1.46–9.70] and \textit{C. showae} (pOR 2.39, 95\% CI 1.11–5.18) were associated with increased odds of IBD.\textsuperscript{27} There were suggestive positive trends for \textit{C. hominis} (pOR 1.58, 95\% CI 0.91–2.75) and \textit{C. ureolyticus} (pOR 2.34, 95\% CI 0.77–7.16), while \textit{C. jejuni}, \textit{C. rectus}, and \textit{C. gracilis} were not associated with odds of IBD, CD or UC.\textsuperscript{27} Salmonella species. In the same nationwide registry-based cohort study from Denmark with over 94 million patient-years of follow up, there were a total of 41,628 stool cultures that were positive for \textit{Salmonella} species. An episode of gastroenteritis with stool culture positive for \textit{Salmonella} species was significantly associated with increased risk of new-onset UC (IRR 3.0, 95\% CI 2.6–3.4) and CD (IRR 2.2, 95\% CI 1.7–2.7) compared with patients without gastroenteritis.\textsuperscript{16} The more recent nationwide case-control study from Sweden of 480,721 patients also cited above similarly demonstrated a positive association between a diagnosis of \textit{Salmonella} and likelihood of IBD, albeit with effect estimates of slightly lower magnitude of effect (aOR for UC 1.49, 95\% CI 1.15–1.94; aOR for CD 1.82, 95\% CI 1.26–2.62).\textsuperscript{18} Enterohpatic \textit{Helicobacter} species. Enterohpatic \textit{Helicobacter} species (EHS) including \textit{Helicobacter fennelliae} (\textit{H. fennelliae}), \textit{H. cinaedi}, and non-\textit{H. pylori}-like (non-HPL) strains have been implicated in the development of clinical IBD. In the largest association study of \textit{Helicobacteraceae} DNA and IBD, which included intestinal biopsies from 77 patients with CD and from 102 patients without IBD, non-HPL EHS were detected significantly more often in the intestinal biopsies of patients with CD compared with non-IBD controls (23\% versus 12\%, \textit{p} = 0.04).\textsuperscript{28} A meta-analysis

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Pathogens} & \textbf{Increased risk} & \textbf{Decreased risk} \\
\hline
\textbf{Bacteria} & \textbf{Salmonella species} & \textbf{Helicobacter pylori} \\
& \textbf{Escherichia coli} &  \\
& \textbf{Yersinia enterocolitica} &  \\
& \textbf{Campylobacter species} &  \\
& \textbf{Enterohpatic Helicobacter species} &  \\
& \textbf{Mycobacterium avium paratuberculosis} &  \\
& \textbf{Clostridioides difficile} &  \\
& \textbf{Listeria monocytogenes} &  \\
& \textbf{Proteus mirabilis} &  \\
& \textbf{Klebsiella pneumoniae} &  \\
& \textbf{Citrobacter species} &  \\
\hline
\textbf{Viruses} & \textbf{Norovirus} &  \\
& \textbf{Cytomegalovirus} &  \\
& \textbf{Epstein–Barr virus} &  \\
& \textbf{Human herpes virus 3} &  \\
& \textbf{Human herpes virus 6} &  \\
& \textbf{Human herpes virus 8} &  \\
& \textbf{Measles virus} &  \\
& \textbf{Mumps virus} &  \\
& \textbf{Rubella virus} &  \\
& \textbf{Rotavirus} &  \\
& \textbf{Adenovirus} &  \\
\hline
\textbf{Fungi} & \textbf{Candida species} &  \\
& \textbf{Aspergillus species} &  \\
& \textbf{Cryptococcus neoformans} &  \\
\hline
\textbf{Parasites} & \textbf{Amoeba/Entamoeba histolytica} & \textbf{Trichuris suis} \\
& \textbf{Toxoplasma gondii} & \textbf{Hymenolepis diminuta} \\
& \textbf{Schistosoma species} & \textbf{Nector americanus} \\
\hline
\end{tabular}
\caption{Specific pathogens associated incident IBD.}
\end{table}
of nine case-control studies (545 patients with IBD and 524 non-IBD controls) confirmed these findings and demonstrated a pooled 2.6-fold higher odds (pOR 2.62, 95% CI 1.48–4.63) of IBD among individuals exposed versus non-exposed to non-HPL EPS.27 Notably, in this same study, a secondary meta-analysis of 15 studies totaling 906 patients with IBD and 758 patients without IBD demonstrated a statistically non-significant positive association between EHS and likelihood of IBD (pOR 1.51, 95% CI 0.95–2.41), as well as no difference in the odds of IBD among HPL-EHS exposed versus non-exposed (pOR 1.14, 95% CI 0.54–2.42).27 However, publication bias, delayed recognition of non-HPL-EHS, and heterogeneity may confound interpretation of these studies. The diagnostic tools used to detect non-HPL-EHS are likewise relevant, given that there is molecular overlap with HPL-EHS and Campylobacter species.

*Mycobacterium avium paratuberculosis.* There are over 65 studies analyzing the association between Mycobacterium avium paratuberculosis (MAP) and IBD, especially CD. Despite being one of the most frequently studied pathogens in IBD, the clinical relevance of MAP in IBD pathogenesis remains controversial.29 Part of the controversy derives from inconsistent methods of MAP detection, specifically culture techniques, which have many limitations including poor sensitivity, requirement for specialized media, and a long incubation time. By contrast, PCR-based testing allows for a more standardized method of detection and has now supplanted culture techniques. PCR-based testing of IS900 – a multi-copy insertion element of MAP – is considered the most rigorous diagnostic method for MAP detection. Although a relatively new technique, the handful of studies that utilize these more refined PCR techniques support an association between MAP and CD.

In a cross-sectional study of 100 patients with CD, 100 with UC, and 100 controls without IBD, PCR testing was positive for the IS900 MAP insertion element in 52% of biopsies from patients with CD compared with 2% of UC and 5% of controls (p<0.001).30 In a similarly designed study of 22 patients with CD, 20 with UC, 21 with aphthous ulcers of the terminal ileum, and 42 non-IBD controls, the prevalence of MAP based on IS900 assays was significantly higher in patients with CD compared with controls without IBD (29% versus 0%, p = 0.02).31 However, the prevalence of MAP was not significantly different between patients with UC (18%) or ileal aphthous ulcers (12%), although insufficient power due to small sample size is a consideration.31

Conversely, several studies utilizing culture and alternative PCR technologies (i.e., non IS900 PCR) have not demonstrated a significant association between MAP and CD.32–35 Collectively, these conflicting conclusions suggest that MAP may play a role in the pathogenesis or disease course of CD in a subset of individuals. However, optimal techniques for MAP identification and the causal role of MAP in IBD pathogenesis remain to be clarified.

*Clostridioides difficile.* There are numerous studies analyzing the association between *C. difficile* infection (CDI) and IBD. Very few studies, however, have analyzed CDI as a risk factor for new onset IBD, as it is challenging to discriminate reverse causality or the innocent bystander effect of CDI in IBD. In the aforementioned nationwide case-control study of 480,721 patients from Sweden, the largest study analyzing the association between CDI and IBD, a diagnosis of *C. difficile* was associated with higher odds of UC (aOR 4.02, 95% CI 2.94–5.49) and CD (aOR 4.25, 95% CI 2.79–6.47).18 One exploratory study of patients with CDI-associated diarrhea compared IgG-specific humoral immune response with *C. difficile* toxins A and B between 10 patients with concomitant IBD and 19 patients without IBD.36 While levels of circulating IgG antibodies to *C. difficile* toxins A and B were not significantly different between groups, IgG antibodies to *C. difficile* toxins A and B were detected on culture of large and small intestinal mucosal samples only in subjects with IBD.36 This finding is significant, as none of the patients with IBD had a history of clinical CDI, suggesting previous asymptomatic exposure to toxigenic *C. difficile.*36 High prevalence of toxigenic *C. difficile* carriage in patients with IBD is supported by several investigations,37–39 although the clinical impact is not clear.

While the reduced diversity of bacterial species in IBD may predispose to *C. difficile* colonization, emerging data also implicate genetic factors. In one study of 319 patients with UC, genetic risk alleles explained a larger portion of the variance in CDI risk than clinical factors such as antibiotic
exposure, suggesting that host immunity may play an important role in susceptibility to CDI. The data provide some support that there may be a true contributory role of *C. difficile* in IBD pathogenesis, at least among genetically predisposed individuals.

*Listeria monocytogenes*. Few studies have examined the role of *L. monocytogenes* in IBD, either as a non-specific antigenic stimulus or as a specific pathogen trigger. In one non-comparative study of patients with CD, there was a positive antibody response to *L. monocytogenes* in 75% of intestinal and mesenteric lymph node specimens, with immunolabeled macrophages and giant cells distributed underneath ulcers, along fissures, around abscesses, within the lamina propria, in granulomas, and in the germinal centers of mesenteric lymph nodes. However, in a subsequent study of 274 colonoscopic biopsies, *L. monocytogenes* DNA was similarly detected in intestinal biopsies of both patients with IBD and in non-IBD controls. This underscores the widespread presence of this organism in the environment and brings into question the direct etiopathogenic role of *L. monocytogenes* in IBD pathogenesis.

*Yersinia* species. In the aforementioned nationwide case-control study of 480,721 patients from Sweden, a diagnosis of *Yersinia enterocolitica* was associated with higher odds of CD (aOR 9.59, 95% CI 3.04–30.3), but not UC (aOR 2.61, 95% CI 0.87–7.83). In a case series of 54 intestinal resection specimens from 52 patients with CD, 40 normal bowel specimens, 30 cases of acute appendicitis, and 50 cases of other non-IBD active colitides, *Yersinia* DNA, including *Y. pestis*, *Y. paratuberculosis*, and *Y. enterocolitica*, was identified in 31% of CD specimens, but in 0% of the specimens obtained from the other groups. In a similarly designed study that tested colonic specimens from 77 children with Crohn’s colitis, 45 children with UC, and 10 children who underwent appendectomies, *Yersinia* species were detected significantly more often in specimens from children with CD compared with all other groups (9% versus 0%; *p* = 0.006). However, a recent cross-sectional study of 470 ileal samples from 262 CD patients and 76 non-IBD controls demonstrated discrepant findings, as *Yersinia* species were detected in 10% of CD patients and 12% of controls (*p* > 0.05). While none of these studies established causality, there is suggestion of shared genetic predisposition since loss-of-function mutations in Nucleotide Oligomerisation Domain 2 (NOD2) – a key gene of innate immunity – are associated both with CD as well as resistance to *Yersinia*. Some have hypothesized that some NOD2 mutations may have been selected for through evolution, specifically during past plague outbreaks.

**Viruses**

Evidence for viruses as risk determinants of IBD among susceptible people is limited. The viruses most studied are those for which testing modalities are readily available. To this end, most studies to date have investigated species within the herpesvirus family, including cytomegalovirus (CMV), EBV, and human herpes virus 6 (HHV-6).

In one study of 79 IBD patients (47 UC and 32 CD) and 15 non-IBD controls, CMV and HHV-6 immunohistochemistry intensity and coexistence correlated with endoscopic disease severity, endoscopic activity, and number of immunosuppressive therapies. In another study of 84 patients with IBD and 115 non-IBD controls, EBV DNA load by immunohistochemistry in peripheral blood mononuclear cells was higher in patients with IBD, who consequently also showed high levels of lytic and latent EBV gene expression localized to proliferating B-lymphocytes; this was particularly pronounced among patients not responding to IBD therapy (*p* < 0.05). However, because there is nearly universal exposure to EBV by adulthood, strong conclusions from association studies of EBV and IBD, especially among adults, are limited at best. For example, one cross-sectional study of EBV seroprevalence in 263 IBD patients demonstrated that EBV seronegativity was similar to the general, with seropositivity approaching 100% after age 25 years, irrespective of IBD status. It is generally unclear why EBV-associated diseases such as mononucleosis and lymphoproliferative disorders occur in some individuals and not others. While not yet investigated, one possibility is that the timing of EBV exposure is relevant, such that EBV exposure in the early life years during the critical phases of immune development modifies IBD risk among genetically susceptible individuals.

With respect to HHVs more broadly, one study analyzed 41 patients with IBD, and reported that EBV, CMV, but not of herpes simplex virus (HSV)-1, HSV-2, Varicella zoster virus (VZV), HHV-6,
HHV-7, or HHV-8, were more commonly identified in the colonic mucosa of individuals with IBD than without IBD (EBV: 54% versus 23%, \( p=0.003 \); CMV: 24% versus 8%, \( p=0.0001 \)).

Norovirus is one non-HHV that has been investigated as a risk determinant in IBD pathogenesis. However, data are limited and there is the consideration of selection bias as well given that norovirus is tested for in the setting of a clinical presentation of diarrhea. That being said, in the nationwide case-control study from Sweden already cited, a diagnosis of norovirus remained independently associated with higher odds of CD (aOR 3.19, 1.28–7.96), but not UC (aOR 1.74, 95% CI 0.85–3.57), after adjusting for several factors including sex, age, birth year, place of residence, previous gastrointestinal surgery, autoimmune disease, and family history of IBD.

Based on these data, it remains unclear whether norovirus, CMV, EBV, and HHV-6 are involved in the pathogenesis of disease, associated with disease flares, complications, and response to therapy, or are simply innocent bystanders of active disease (or immunosuppression) and instead more reflect the baseline high prevalence in the general population. Well-designed prospective studies with adequate control populations are needed and will also help to define the impact of timing of exposure and other modifying factors.

**Fungi**

Technologic advancements have enabled deeper characterization of previously overlooked members of the gut microbiome that might also play a critical role in IBD pathogenesis and its disease course. Fungi fall into this category, but the same issues of causality and innocent bystander remain. Recent studies using advanced sequencing approaches demonstrate that fungal diversity is altered in ileal and colonic biopsies from patients with CD compared with healthy controls. Specifically, multiple studies have demonstrated varying proportions of gastrointestinal pathogenic Candida species, Malassezia species, Aspergillus species, and Cryptococcus neoformans. While studies on UC have been somewhat inconsistent, altered fungal diversity has still been reported. While the complex interplay and directionality between the gut mycobiome, fungal pathogens, and mucosal inflammation remain poorly understood, these findings do suggest specific fungi might be relevant in disease pathogenesis. Further studies are required, including studies of genetic and other susceptibility determinants.

**Gastrointestinal pathogens and attenuated risk of IBD**

Many population-based, cross-sectional, and retrospective studies have demonstrated a protective association between \textit{H. pylori} or intestinal helminth colonization with IBD.

**Bacteria**

\textit{Helicobacter pylori}. \textit{H. pylori} is still the most common chronic bacterial infection worldwide, with over half the global population estimated to be colonized or infected; however, the prevalence of \textit{H. pylori} has been decreasing over the past several decades, due largely to industrialization, less crowding, and improved living conditions, as well as targeted eradication therapy. Decreases in \textit{H. pylori} prevalence on a population level correspond to an increase in immune-mediated diseases, particularly CD, and is supported by many epidemiological studies. At least in endemic areas, \textit{H. pylori} exposure most often occurs early in life, and, depending on host susceptibility factors and host-microbial interactions, might qualify as a “critical early exposure” given the downstream consequences of immunomodulatory effects and alterations of the gastric, oral, and colonic microbiome associated with \textit{H. pylori} colonization/infection.

To date, five meta-analyses of the association between \textit{H. pylori} exposure and IBD have been published. The most recent and most comprehensive meta-analysis by Castano-Rodriguez and colleagues reported that, among 40 case-control studies with 6130 IBD cases and 74,659 non-IBD controls, \textit{H. pylori} exposure was associated with a 57% lower odds of IBD (pOR 0.43, 95% CI 0.36–0.50), including CD (pOR 0.38, 95% CI 0.31–0.47), UC (pOR 0.53, 95% CI 0.44–0.65), and IBD-U (pOR 0.43, 95% CI 0.23–0.80). Regardless of age and geography, the inverse association was maintained, and was stronger for pediatric (pOR 0.24, 95% CI 0.14–0.43) versus adult (pOR 0.45, 95% CI 0.38–0.53) populations and Eastern (pOR 0.35, 95% CI 0.26–0.48) versus Western (pOR 0.46, 95% CI 0.38–0.55) populations, albeit not statistically significantly. The
other meta-analyses, one of which included only Asian studies,68 reported consistent findings, with all meta-analyses demonstrating a significant inverse association between \textit{H. pylori} and IBD that was generally more pronounced for CD versus UC. \textit{H. pylori} strain-specific constituents might mediate, at least in part, this protective association, plausibly through immunomodulation. One recent meta-analysis of 1748 people (688 with CD, 272 with UC) found that, compared with CagA-negative \textit{H. pylori} exposure or \textit{H. pylori} non-exposure overall, exposure to CagA-positive \textit{H. pylori} was associated with a significantly lower odds of IBD (pOR 0.31, 95% CI 0.21–0.44) and CD (pOR 0.23, 95% CI 0.15–0.35), with a suggestive trend for UC (pOR 0.66, 95% CI 0.34–1.27).71 Interestingly, there was no significant difference in the odds of IBD overall, CD, or UC between \textit{H. pylori} exposed, CagA seronegative, and \textit{H. pylori} non-exposed individuals.

It is important to emphasize that not all Helicobacter species are inversely associated with IBD. As detailed in the sections above, the meta-analysis by Castano-Rodriguez and colleagues also analyzed the association between EHS and IBD, stratified by non-HPL-EHS and HPL-EHS.

**Parasites**

\textit{Trichuris species}. Helminths play an important immunoregulatory role in mucosal immunity and the gut microbiome. Similar to the epidemiology of \textit{H. pylori} exposure, intestinal helminthic infections are more common in regions with higher population densities, less industrialization, and poorer water sanitation, which tend to also be regions of lower IBD prevalence. A handful of studies have suggested that the absence of intestinal helminths is associated with an increased likelihood of new onset IBD.72–74 Of the limited literature, the inverse association between \textit{Trichuris} species and IBD is perhaps most described.75 One small case-control study of 151 South African patients with IBD and 219 controls without IBD reported that, after adjusting for age and sex, \textit{Trichuris} helminth exposure versus non-exposure was associated with lower odds of IBD (aOR 0.2, 95% CI 0.1–0.4).76 These data, however, were based on self-reported infection early in life in a region where helminth infections are endemic. Indeed, this study also highlights the difficulty of conducting rigorous epidemiological and clinical studies in certain regions related to insufficient resources and infrastructure for exposure assessment and accurate case confirmation.

Few studies have sought to define mechanisms underlying these observations. Notably, based on the limited but biologically plausible evidence for a protective effect of intestinal helminths in IBD, several studies have evaluated the efficacy (and safety) of the pig whipworm \textit{Trichuris suis} for the treatment of IBD with mixed results.77–82 Many confounders, measured and unmeasured, among other study limitations limit strong conclusions, however, and more data are needed.

**Gastrointestinal pathogens in relapse of IBD**

Gastrointestinal infections are commonly implicated in relapse (flares) of already established IBD (Table 2).83–88 There is considerable overlap between the clinical presentation of enteric infection and IBD flare, suggesting that these conditions may be mutually exclusive or co-exist, further complicating interpretation and causal determination. Current clinical guidelines recommend testing for \textit{C. difficile} in all patients with IBD who have worsening or new onset diarrhea and testing for CMV in patients with severe active IBD, particularly if there is concomitant steroid use and disease refractory to medical therapy. Otherwise, there is no consensus for testing for other gastrointestinal pathogens in the setting of an IBD flare. The clinical significance of a positive result for non-\textit{C. difficile} enteric infection in patients with active IBD is debated due to lack of robust outcomes-based evidence.

**\textit{Clostridioides difficile}**

Multiple studies have demonstrated consistently that, among patients with IBD, those who have confirmed CDI have more pronounced dysbiosis and significantly worse clinical outcomes, such as longer hospital stays, higher colectomy rates, higher recurrence rates, and increased mortality, compared with those without CDI.83,89–100 Moreover, established risk factors for CDI such as nosocomial acquisition, age, and recent antibiotic use may not be significant risk factors for CDI in patients with IBD.94,101–103 One study of 461 consecutive patients hospitalized for IBD flares, detected toxigenic \textit{C. difficile} and non-toxigenic \textit{C. difficile} in 35 (8%) and 10 (2%) hospitalized patients, respectively.104
Similarly designed study that instead used multiplex PCR, of 214 patients with IBD who underwent 295 multiplex stool PCR tests for an IBD flare, toxigenic C. difficile was the most common pathogen identified \((n=38, 13\%\) among 79 \((27\%)\) tests where a pathogen was detected.\(^8^3\) Notwithstanding, there is controversy regarding the diagnosis and clinical relevance of C. difficile in IBD disease course. Based on the cross-sectional studies referenced above, patients with IBD have a high prevalence of asymptomatic C. difficile colonization, which similarly precludes major conclusions regarding the contribution of CDI to IBD flares.

**Non-Clostridioides difficile enteric infections**

The introduction of multiple PCR testing has allowed for more widespread detection of other enteric pathogens which may or may not be players in IBD flares. In the study cited above that included 214 patients with IBD who underwent 295 multiplex stool PCR tests during a flare, after C. difficile, the most commonly detected microbes were E. coli subtypes \((8\%)\) and viruses \((5\%)\).\(^8^3\) Patients who tested negative for an enteric infection were more likely to have IBD medications added or up-titrated \((49\% \text{ versus } 29\%, p=0.027)\), suggesting that the results of enteric testing impacted IBD management.\(^8^3\) Focusing on culture data, other studies have demonstrated worse outcomes, including higher rates of colectomy and mortality, in patients with IBD flare complicated by Campylobacter and Salmonella.\(^1^0^5,1^0^6\)

More recently, a cross-sectional study analyzed 577 patients with IBD \((277 \text{ CD, } 300 \text{ UC})\) flare and 8826 unmatched control subjects without IBD who had multiplex stool PCR tests ordered during a diarrheal illness. Compared with patients without IBD, patients with IBD were significantly less likely to test positive overall \((\text{CD } 18.1\%\text{, UC } 16.1\%, \text{ no IBD } 26.6\%, p<0.001)\), but it is important to note that not all microbes tested for with the stool PCR test are necessarily pathogenic \((\text{e.g., parasites})\).\(^1^0^7\) As such, there were some notable differences based on the specific pathogen. Compared with patients without IBD, patients with CD tested positive more often for Campylobacter \((13.9\% \text{ versus } 7.6\%, p=0.01)\), Plesiomonas \((2.6\% \text{ versus } 0.7\%, p=0.049)\), and E.coli subtypes \((64.3\% \text{ versus } 47.6\%, p<0.001)\), including Enteropathogenic E.coli \((20.9\% \text{ versus } 13.5\%, p=0.03)\), and Enteropathogenic E.coli \((33.9\% \text{ versus } 22.1\%, p=0.004)\), but less often for parasites \((0.9\% \text{ versus } 6.1\%, p=0.01)\) and norovirus \((7.8\% \text{ versus } 17.4\%, p=0.02)\).\(^1^0^7\) When comparing IBD subtypes, patients with UC less often tested positive for viruses compared with patients with CD \((16.5\% \text{ versus } 32.8\%, p=0.004)\), specifically norovirus \((7.8\% \text{ versus } 24.6\%, p<0.001)\).\(^1^0^7\)

| Table 2. Specific pathogens associated flare of prevalent IBD. |
|-------------------------------------------------------------|
| **CD**                                                      |
| Bacteria                                                   |
| + Campylobacter species                                    |
| + Clostridioides difficile                                  |
| + Enteropathogenic Escherichia coli                         |
| Viruses                                                    |
| + Norovirus                                                |
| Parasites                                                  |
| - Giardia lambia                                           |
| - Cryptosporidium                                          |
| - Cyclospora cayetanensis                                  |
| - Entamoeba histolytica                                    |
| **UC**                                                     |
| Bacteria                                                   |
| + Campylobacter species                                    |
| + Plesiomonas shigelloides                                 |
| + Enteropathogenic Escherichia coli                         |
| Viruses                                                    |
| - Norovirus                                                |
| Parasites                                                  |
| - Giardia lambia                                           |
| - Cryptosporidium                                          |
| - Cyclospora cayetanensis                                  |
| - Entamoeba histolytica                                    |

+, Increased cross-sectional prevalence during flare compared to symptomatic patients without IBD.
- Decreased cross-sectional prevalence during flare compared to symptomatic patients without IBD.
CD, Crohn’s disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.
that both asymptomatic and symptomatic patients were included, possibly even further diluting the ability to detect a true difference. In a retrospective study of 1345 patients with IBD flare who underwent stool PCR or culture for non-\textit{C. difficile} bacterial pathogens, only 25 tests (2\%) were positive. Moreover, a higher proportion of patients with non-\textit{C. difficile} bacterial infections were in remission of their IBD within 1 year compared with patients with \textit{C. difficile} infection or non-infectious flare.\textsuperscript{109} This study, however, used mixed diagnostic methods and did not evaluate for enteric viruses.

Separately, CMV has been detected in approximately 30\% cases of steroid-refractory IBD flares.\textsuperscript{110–112} Similar to many studies of other enteric infections, the variable study designs, study populations, diagnostic methods, and testing/indication biases, to name a few, compromise the ability to conduct rigorous analyses and discern true pathogenic role. CMV reactivation is common in patients with moderate to severe colonic disease especially with concomitant steroid use, with a reported prevalence of 5–17\%, and as high as 25–30\% among patients requiring colectomy for severe colitis.\textsuperscript{113} The clinical relevance of CMV infection as opposed to just a bystander detected in the setting of severe disease and immunosuppression is not clear, and most often depends on the clinical scenario. In one study of 69 patients with moderate-to-severe UC who were tested for CMV reactivation every 2 weeks for 8 weeks using the CMV antigenemia assay and serum quantitative real-time PCR assay, clinical outcomes, including rates of remission and colectomy, were not significantly different among the CMV reactivation-positive versus negative patients.\textsuperscript{114} Other serologic studies have demonstrated similar findings, arguing against CMV as a major pathogen in IBD relapse.\textsuperscript{115,116} However, latent or subclinical CMV does not correlate with CMV colitis which requires colonic tissue for diagnosis and these negative studies employed variable non-tissue diagnostics methods.

As with all gastrointestinal infections complicating flares of IBD, in the absence of serial, longitudinal, prospective testing and an appropriate reference group (e.g., patients without IBD with diarrhea or other GI symptoms), it is difficult to conclude that specific enteric pathogens are causative factors in disease flares or whether they represent a surrogate marker of disease severity or susceptibility to pathogen acquisition. In addition, very little is known regarding the influence of specific enteric pathogens on IBD outcomes and disease progression. Despite these and other limitations, further investigation would be valuable given the potential role of enteric pathogens in IBD incidence, disease flares, and complications.

**Discussion**

In summary, specific gastrointestinal infections including bacteria, viruses, parasites, and fungi, may modify the risk of developing IBD and trigger or complicate flares in patients with established IBD. Critical study design limitations and biases preclude the ability to definitively establish a direct causal relationship between gastrointestinal infection or colonization and new onset or flare of IBD. As such, the possibility of reverse causality, IBD increasing the risk of infection or colonization acquisition, and that these species are merely “innocent bystanders” remains unsettled and complicates our understanding of IBD. Specifically, \textit{C. difficile} carriage occurs more often in the setting of reduced diversity of intestinal microbial species that occurs following antibiotic exposure, with reduced diversity predisposing to \textit{C. difficile} colonization with or without clinical infection. This observation further complicates our understanding of its role in IBD pathogenesis and contributes to the reverse causality consideration, since reduced diversity might predispose to IBD, and reduced diversity also occurs as a result of IBD. Antibiotic exposures are likely also to modify or confound these associations. Nonetheless, clinical data described above have demonstrated a lower overall pathogen detection rate in patients with an exacerbation of existing IBD compared with non-IBD controls, the reasons for which are unclear. These data suggest that while patients with IBD have an impaired intestinal barrier with limited or dysfunctional antibacterial activity, this deficit does not directly translate into a broadly increased risk of gastrointestinal pathogen acquisition or infection. Thus, the above clinical data provide a wealth of insight and hypotheses for our understanding of factors driving the preclinical and clinical phases of IBD.

In addition to the above clinical associations, experimental and translational data offer a window into whether these clinical associations have mechanistic plausibility in IBD pathogenesis. Our group recently reviewed translational data implicating
certain gastrointestinal pathogens in incident IBD\textsuperscript{15}; herein, we highlight relevant mechanisms to provide context for the clinical evidence detailed above, but we encourage interested readers to refer to that more comprehensive review. In genetically susceptible individuals, specific pathogens and the associated downstream consequences might cause gut dysbiosis, exacerbate existing dysbiosis, induce immunological scar and deleteriously alter immune responses, or might activate immune responses \textit{via} direct damage the intestinal mucosa; each of these mechanisms, alone and in concert, might trigger the clinical presentation of new IBD and flare of existing disease.\textsuperscript{15} Moreover, virulence mechanisms involved in microbial fitness can directly or indirectly damage the epithelial barrier, triggering the polarization of lymphocytes and myeloid cells towards an inflammatory state of activation in an attempt by the body to remove the offending pathogen.

A defective intestinal barrier is a major feature of IBD and infectious disease. A recent study demonstrated intestinal permeability precedes a diagnosis of CD, raising the possibility that a pathogen-induced disruption of the barrier is a contributing factor.\textsuperscript{117} Experimental systems, including those that seek to explain the link between certain genes and IBD risk, offer potential mechanisms. Abnormal microbial-sensing by pattern-recognition receptors, a compromised mucin barrier, impairment in autophagy, diminished functional antimicrobial activity, direct epithelial cell damage, and changes in tight junction permeability, all contribute to loss of intestinal barrier integrity.\textsuperscript{118} In the setting of reduced protective host-defense mechanisms and dysbiosis, there is greater commensal and pathogenic microbial contact with the intestinal epithelium, which is associated with aberrant mucosal immune responses that represent the hallmark of IBD. Conversely, pathogens such as \textit{Helicobacter} and certain helminths, and their downstream products, might reduce dysbiosis and/or counteract inflammatory pathways, preventing IBD onset or flare of existing disease. The balance between pro- and anti-inflammatory microbes, and whether the individual is genetically predisposed to mounting a vigorous immune response, may determine the likelihood of developing IBD or triggering relapse of disease.

In terms of gastrointestinal pathogens and IBD onset, in addition to issues with establishing causality, many of the above studies analyzed subset species as a composite genus group. However, this may dilute the observed effect for species-specific pathogens and shroud clinically relevant differences. Other limiting factors include an inability to detect prior events, as PCR and culture techniques do not account for lifetime exposure to various gastrointestinal pathogens. The multitude of enteric infections, as well as the timing (\textit{e.g.} early \textit{versus} later in life), underlying genetic susceptibility, and change in the gut microbiome, rather than a singular pathogen, may be most relevant, particularly when considering the complexity of IBD pathogenesis. In fact, limited data has suggested gastroenteritis later in life is linked more strongly to an increased risk of IBD, or perhaps it is the lack of exposure earlier in life that may influence immune tolerance and increase the risk for subsequent IBD.\textsuperscript{18} Thus, timing of exposure to a gastrointestinal pathogen is important and more data are needed.

Overcoming selection bias in observational, associative studies investigating the role of gastrointestinal pathogens in IBD flares is also challenging, since testing for enteric infections is generally limited to those with an exacerbation in symptoms and is not routine for asymptomatic patients, thus limiting a true “control” population. Moreover, patients with IBD are more often tested for gastrointestinal pathogens compared with patients without IBD, since the identification of an enteric pathogen in a patient with IBD may directly impact clinical decision making to a greater degree.\textsuperscript{83,109} Although the lack of robust data, the heterogeneity of diagnostic methods, and inability to achieve complete confounder adjustment contribute to the inability to establish causality for most gastrointestinal pathogens in IBD flares, it is nevertheless conceivable that certain enteric pathogens may trigger relapse of clinical IBD or contribute to attenuated or failed responses to IBD therapies.

Experimental and translational studies provide complementary evidence for the role of enteric infections in IBD pathogenesis by delineating potential pathogenic and protective mechanisms. These mechanisms may be investigated for future preventative and therapeutic interventions in IBD. Further data incorporating deep sequencing technologies will continue to reveal the relationship between enteric pathogens and IBD. Well-designed prospective studies on carefully selected
patients are required to evaluate the clinical implications of enteric pathogens on the risk and course of IBD, and how pathogens may contribute to the efficacy and safety of IBD therapies. A better understanding of these complex gene-microbe-environment interactions will advance our efforts toward IBD prevention and a more personalized approach to disease management.

**Authorship**
Guarantor: Axelrad

Study concept and design: All co-authors.

Acquisition of data: JEA and SCS.

Writing first draft of the manuscript: JEA and SCS.

Critical revision of the manuscript for important intellectual content and approval of final version: All co-authors.

All authors approved the final version of the article, including the authorship list.

**Conflict of interest**
JEA reports receiving research grants from BioFire Diagnostics; consultancy fees or honorarium from BioFire Diagnostics and Janssen; and holds US patent 2012/0052124A1. KC reports receiving research funding from Pfizer, Takeda, and AbbVie; consultancy fees or honorarium from Puretech Health, Genentech, and AbbVie; and holds US patent 10,722,600 and provisional patent 62/935,035. JFC reports receiving research grants from AbbVie, Janssen Pharmaceuticals and Takeda; receiving payment for lectures from AbbVie, Amgen, Allergan, Inc. Ferring Pharmaceuticals, Shire, and Takeda; receiving consulting fees from AbbVie, Amgen, Arena Pharmaceuticals, Boehringer Ingelheim, Celgene Corporation, Celltrion, Eli Lilly, Enterome, Ferring Pharmaceuticals, Genentech, Janssen Pharmaceuticals, Landos, Ipsen, Medimmune, Merck, Novartis, Pfizer, Shire, Takeda, Tigenix, Viela bio; and holds stock options in Intestinal Biotech Development and Genfit.

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**ORCID iDs**
Jordan E. Axelrad https://orcid.org/0000-0003-1951-7790
Shailja C. Shah https://orcid.org/0000-0002-2049-9959

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