Clinical Infectious Diseases

MAJOR ARTICLE

Association of *Mycobacterium Avium Paratuberculosis* With Crohn's Disease: A Large Multicenter Study From a Tuberculosis-Endemic Region

Sudheer Kumar Vuyyuru,[1] Urvashi Singh,[2] Prasenjit Das,[3] Shiv Basant,[1] Vishwajeet Singh,[4] Saroj Kant Sinha,[5] Kusum Sharma,[6] B. S. Ramakrishnan,[7] Kannan Palaniyandi,[8] Rupesh Pokharna,[9] Bharti Malhotra,[10] Shobna Bhatia,[11] Gopal Krishna Dhali,[12] Rajib Sarkar,[12] Charu Sharma,[13,14] Siddhartha Dattagupta,[3] Manjula Singh,[15] Rakesh Kochhar,[5] Sreenivas Vishnubhatla,[16] and Vineet Ahuja[1]

[1]Department of Gastroenterology and Human Nutrition, All India Institute of Medical Sciences, New Delhi, India; [2]Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India; [3]Department of Pathology, All India Institute of Medical Sciences, New Delhi, India; [4]Department of Biostatistics, All India Institute of Medical Sciences, Nagpur, India; [5]Department of Gastroenterology, PGIMER, Chandigarh, India; [6]Department of Microbiology, PGIMER, Chandigarh, India; [7]Department of Gastroenterology, SRM Institute, Chennai, Tamil Nadu, India; [8]Department of Immunology, National Institute for Research in Tuberculosis (ICMR), Chennai, Tamil Nadu, India; [9]Department of Gastroenterology, SMS Medical College, Jaipur, Rajasthan, India; [10]Department of Microbiology, SMS Medical College, Jaipur, Rajasthan, India; [11]Department of Gastroenterology, KEM Medical College, Mumbai, Maharashtra, India; [12]Department of Gastroenterology, IPGMER Kolkata, Kolkata, West Bengal, India; [13]CSIR-Institute of Microbial Technology, Chandigarh, India; [14]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India; [15]Indian Council for Medical Research, ICMR headquarters, New Delhi, India; and [16]Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, India

Background. The causal relationship between Crohn's disease (CD) and *Mycobacterium avium* subspecies *paratuberculosis* (MAP) remains controversial.

Methods. This multicenter observational study, conducted across 7 tertiary care centers in India, enrolled newly diagnosed, treatment-naive patients with CD as cases and treatment-naive patients with intestinal tuberculosis (ITB) or ulcerative colitis (UC) and healthy individuals undergoing sigmoidoscopy for hemorrhoids as controls. MAP detection was performed using serology for MAP antibodies, polymerase chain reaction (PCR), real-time quantitative PCR, and solid/liquid cultures of blood and colonic biopsy specimens. In situ PCR and immunohistochemistry were also applied to paraffin-embedded tissue sections to evaluate the presence of MAP across groups.

Results. A total of 889 participants were recruited (148 with CD, 288 with ITB, 251 with UC, and 202 controls without inflammatory bowel disease) were included. The seropositivity of MAP was significantly higher in patients with CD than in controls (20.6% [13 of 63] vs 16.2% [12 of 74] for healthy controls, 7.8% [9 of 116] for patients with ITB, and 4.8% [4 of 84] for patients with UC; $P < .01$). With tissue PCR analysis using a IS900-specific sequence with colonic biopsy specimens, a significantly higher number of patients with CD were positive for MAP compared with controls (11% [9 of 82] vs 7.1% [5 of 70] for healthy controls, 1% [2 of 188] for patients with UC, and 0.5% [1 of 198] for patients with ITB; $P < .01$). On solid culture of biopsy samples, MAP was detected in 10% of patients with CD (5 of 50), compared with 4.1% (4 of 97) for ITB and 0% (0 of 78) for UC ($P = .02$). However, this difference was not observed with analysis using liquid culture, immunohistochemistry, in situ PCR, or PCR of blood samples.

Conclusions. Our study findings suggest an increased association between MAP and CD. Future studies should explore the possible causal role of MAP in CD and potential therapeutic options to target MAP.

Keywords. Crohn's disease; ulcerative colitis; intestinal tuberculosis; *Mycobacterium avium paratuberculosis*.

Crohn's disease (CD) is a complex immune-mediated condition affecting intestines characterized by diverse manifestations and long-term debilitating complications [1]. The exact etiological agent responsible for CD remains unidentified, but CD is hypothesized to result from a complex interaction between environmental factors, the microbiota, and genetic predisposition. Various microbiological agents including *Listeria monocytogenes*, *Pseudomonas maltophila*, *Mycobacterium kansasii*, *Bacteroides fragilis*, and *Chlamydia pneumoniae*, as well as viral agents such as measles virus and cytomegalovirus have been proposed as potential causes of CD.However, none have been definitively proven due to insufficient evidence [2–4].

The etiological roles of microorganisms such as *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and adherent-invasive *Escherichia coli* remain controversial. MAP is an acid-fast, gram-positive bacillus responsible for Johne disease, an intestinal ileocolonic ulceroconstrictive disease affecting ruminants and causing significant economic losses. In human tissues, MAP exists in a cell wall–deficient form known as a *spheroplast*. Paratuberculosis has a wide geographic distribution and has been reported on almost every continent, in virtually every country engaged in animal agriculture. Herd prevalence of

Received 08 October 2025; accepted 15 December 2025; published online 5 January 2026

Correspondence: V. Ahuja, Department of Gastroenterology and Human Nutrition, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, Delhi, India 110029 (vineet.aiims@gmail.com).

Clinical Infectious Diseases®

https://doi.org/10.1093/cid/ciaf738

MAP in animals ranges from 20% to 70% in the United Kingdom and the United States [5, 6].

The association between MAP and CD was first suggested by Dalziel [7] in 1913, who noted Johne disease–like features in patients with CD. Since then, considerable research has explored this potential link. Although many studies have reported an association between MAP and CD, conclusive evidence supporting a causative role remains lacking. Due to its fastidious and slow-growing nature, isolating MAP in culture is challenging. While molecular techniques such as polymerase chain reaction (PCR) assays have improved detection sensitivity, they cannot distinguish between live and dead organisms. The IS900 genetic sequence, specific to MAP, is commonly targeted in PCR-based identification [8].

MAP is widely prevalent in cattle, and its ability to resist pasteurization and transmit via the fecal-oral route likely explains its high prevalence in the general population. However, factors such as the absence of disease worsening following immunosuppression, lack of response to antitubercular therapy, and no increased incidence of CD among individuals closely working with animals, argue against a direct causal role of MAP in CD. To date, there is no definitive evidence establishing an association between MAP and CD.

There is growing evidence suggesting that treatment strategies directed against MAP may have a role in CD management. A recent phase 3 randomized trial [9]of the MAP directed antibiotic regimen RHB-104 (clarithromycin, 950 mg/d; rifabutin, 450 mg/d; and clofazimine, 100 mg/d) showed improved clinical remission (Crohn's disease activity index <150) at week 26 compared with placebo (in 36.7% vs 22.4%). Newer approaches such as fecal microbial transplantation, vaccines [10], bacteriophages [11], and host-directed therapies [12] are actively being investigated.

The etiological mechanism of MAP in CD has been speculated. Several studies have suggested MAP as an environmental trigger for multiple human autoimmune diseases, such as multiple sclerosis, Type 1 diabetes, CD, and sarcoidosis through molecular mimicry [13, 14]. To date the association between MAP and CD remains an unanswered question. To address this gap, we designed a multicenter, pan-India study to assess the association of MAP using molecular and serological markers in a large cohort of patients with CD, compared with disease controls (patients with intestinal tuberculosis [ITB] or ulcerative colitis [UC]) and apparently healthy controls.

METHODS

Study Objective

The primary objective of this study was to explore the association between MAP with CD using biochemical, molecular, and microbiological methods.

Participants

In this prospective study, blood and stool samples were collected at 6 centers across India (All India Institute of Medical Sciences, New Delhi; Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh; SRM Institutes for Medical Academics, Chennai; KEM Hospital, Mumbai; and SMS Medical College, Jaipur, IPGMER Kolkata). Blood and tissue samples were analyzed at 4 centers (All India Institute of Medical Sciences, New Delhi; SMS Medical College, Jaipur; NIRT, Chennai; and PGIMER, Chandigarh) from August 2016 to May 2019. Ethical approval was obtained from the respective institutional ethical committees. Adult patients (aged ≥18 years) with newly diagnosed and treatment-naive ileocolonic CD were included. Patients with newly diagnosed and treatment-naive UC or ITB were prospectively included as disease controls. Apparently healthy adults undergoing sigmoidoscopy for hemorrhoidal bleeding without previously diagnosed gastrointestinal diseases were considered controls without inflammatory bowel disease (IBD). Patients with HIV infection, previous exposure to steroids, or severe UC, pregnant women, and those not willing to consent were excluded.

Definitions

Diagnosis of CD

The diagnosis of CD was established by the presence of characteristic clinical manifestations and endoscopic, radiological and histological features per consensus guidelines from the European Crohn's and Colitis Organisation [15].

Diagnosis of ITB

The diagnosis of ITB was considered in patients with clinical, radiological, endoscopic, microbiologic, or histopathological features consistent with ITB [16, 17].

Diagnosis of UC

The diagnosis of UC was made per consensus guidelines from the European Crohn's and Colitis Organisation [15].

Healthy Controls

Adult patients with hemorrhoidal bleeding and no other colonic disease undergoing sigmoidoscopy were included as healthy controls.

Sample Collection

Peripheral blood samples (5 mL) were collected in ethylenediaminetetraacetic acid vials for qPCR for MAP DNA. (At 4 centers where culture of buffy coat was done, 10-mL blood samples were obtained.) Serum was separated from 2 mL of blood for enzyme-linked immunosorbent assay (ELISA) for MAP antigens. Six biopsy specimens were taken from an area of active ulceration present in the ileocolonic segment during colonoscopy. Three specimens were preserved in formalin for histopathology, and the remaining 3 were collected in sterile normal saline

for microbiological tests and transported to the laboratory, preferably on ice, as early as possible; chemical preservatives were avoided. The samples were frozen at −70°C until testing. Details about sample collection, processing, and molecular methods are presented in the Supplementary materials.

Detection of MAP

Seropositivity was assessed by detection of antibodies to MAP antigens with ELISA. PCR and real-time quantitative PCR (qPCR) were performed on peripheral blood and colonic biopsy specimens. In situ PCR was performed on formalin-fixed tissue/paraffin-embedded tissue blocks containing adequate tissue. A set of primers (BA5 and BA6) flanking a region of 124 base pairs was synthesized for detection of IS900-specific sequence. Both solid cultures (Lowenstein-Jensen and Herrold's egg yolkmedium) and liquid cultures (MGIT 960 with or without mycobactin J supplement) were performed for MAP. In addition, immunohistochemistry (IHC) staining was performed on paraffin-embedded sections of colonic tissue slides. Further details of techniques are presented in the Supplementary materials.

Statistical Analysis

Data were summarized as frequency (percentage) for categorical variables and mean (SD) or median (interquartile range) for continuous variables, and χ^2 tests were used to compare differences in proportions among different groups. Differences were considered significant at 2-way $P < .05$.

Ethical Clearance

Informed signed consent was obtained from participants. Approval was granted from the respective ethical clearance committees for all centers (IEC/NP-452/12.12.2014).

RESULTS

A total of 889 participants were recruited from 7 centers across India (148 with CD, 288 with ITB, 251 with UC, and 202 healthy control).

Baseline Characteristics of Included Participants

Baseline characteristics of study participants are presented in Table 1. Among the participants 59.9% (n = 533) were male, and 46.3% (n = 412) of participants had a rural background. The median disease duration for participants with CD or UC was 12 months, and the median symptom duration in participants with ITB was 7 months (interquartile range, 3–12 months). The most common disease location for both patients with CD and those with ITB was ileocolonic. The predominant symptom in participants with CD or ITB was abdominal pain (in 73% and 85%, respectively). Diarrhea, blood in stools, and extraintestinal manifestations were more frequent in participants with CD than in those with ITB, whereas, weight loss, loss of appetite, fever, and intestinal obstructive symptoms were more frequent in participants with ITB (Table 2). Among the histological features, ulceration, cryptitis, crypt abscess, and crypt branching were more frequent in CD than in ITB, whereas granulomas were more frequent in ITB.

Detection of MAP With Serological Methods

The seropositivity of MAP, assessed by the presence of antibodies to MAP antigens in peripheral blood, was 20.6% (13 of 63) in CD, compared with 16.2% (12 of 74) in non-IBD controls, 7.8% (9 of 116) in ITB, and 4.8% (4 of 84) in UC. The differences among all groups were statistically significant ($P < .01$) (Table 3).

Table 1. Baseline Characteristics of Included Study Participants

Characteristic	Participants, No. (%)[a]			
	CD (n = 148)	UC (n = 251)	ITB (n = 288)	Healthy Controls (n = 202)
Male sex	87 (58.7)	158 (62.9)	160 (55.5)	128 (63.4)
Rural background	59 (39.8)	131 (52.1)	134 (46.5)	88 (43.3)
BMI, mean (SD)[b]	24.2 (6)	24.4 (6)	22.2 (5)	24.9 (5)
Age at onset, mean (SD), y	36.2 (13)	34.2 (12)	33.5 (14)	…
Vegetarian diet	48 (32.4)	97 (38.6)	110 (38.1)	47 (23.1)
Disease/symptom duration, median (IQR), mo	12 (5–24)	12 (6–24)	7 (3–12)	…
Socioeconomic status, mean (SD)	13.22 (7.35)	14.01 (5.77)	13.66 (5.98)	11.15 (6.58)
Current smoker	4 (2.7)	12 (4.7)	14 (4.8)	4 (1.9)
Current alcohol consumer	4 (2.7)	9 (3.6)	4 (1.4)	1 (0.5)
Segment of bowel involved				
Ileum	33 (24.3)	0 (0)	46 (17.1)	…
Colon	41 (30)	251 (100)	85 (31.6)	…
Ileocolonic	48 (35.3)	0 (0)	126 (46.8)	…

Abbreviations: BMI, body mass index; CD, Crohn's disease; IQR, interquartile range; ITB, intestinal tuberculosis; SD, standard deviation; UC, ulcerative colitis.

[a]Data represent no. (%) of participants unless otherwise specified.

[b]BMI calculated as weight in kilograms divided by height in meters squared.

Table 2. Comparison Between Patients With Crohn's Disease and Patients with Intestinal Tuberculosis

Parameter	Patients, No. (%)[a]		*P* Value
	CD (n = 148)	ITB (n =288)	
Age at onset, mean (SD), y	36.2 (13)	33.5 (14)	.07
Duration of symptoms, median (IQR), mo	12 (5–24)	7 (3–12)	.003
Clinical symptoms			
Diarrhea	96 (65)	107 (37)	<.001
Constipation	15 (10)	43 (15)	.16
Abdominal pain	108 (73)	245 (85)	.002
Blood in stools	77 (52)	47 (16.3)	<.001
Obstruction	8 (5.4)	50 (17.4)	<.001
Fever	43 (29)	111 (38.5)	.050
Weight loss	77 (52)	192 (66.7)	.003
Loss of appetite	43 (29)	126 (43.8)	.003
Extraintestinal manifestations	31 (22.8)	24 (9.2)	<.001
Histopathological features			
Ulceration	85 (63.43)	146 (55.73)	<.001
Cryptitis	60 (44.78)	107 (41.00)	<.001
Crypt abscess	40 (29.85)	67 (25.57)	<.001
Crypt branching	27 (20.15)	37 (14.12)	<.001
Goblet cell depletion	17 (12.69)	39 (14.89)	<.001
Paneth cell metaplasia	6 (4.48)	4 (1.53)	.30
Granuloma	8 (5.97)	62 (23.66)	<.001
Caseation	0 (0.00)	2 (1.01)	.07
Laboratory values, mean (SD)			
ESR (mm/hr)	33.00 (17.75)	40.25 (25.96)	.001
TLC (cells/microliter)	7848.87 (3149.29)	7945.06 (5012.35)	.82
Hemoglobin (gm/L)	10.84 (2.36)	11.21 (1.95)	.14
CRP (mg/L)	24.51 (32.25)	27.69 (43.50)	.002

Abbreviations: CD, Crohn's disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range; ITB, intestinal tuberculosis; SD, standard deviation; TLC, total leukocyte count.

[a]Data represent no. (%) of participants unless otherwise specified.

Table 3. Detection of *Mycobacterium avium* subspecies *paratuberculosis* With Various Methods

Method of Detection	Study Participants, No./Total (%)				*P* Value
	Controls (n = 202)	CD (n = 148)	UC (n = 251)	ITB (n = 288)	
ELISA (blood)	12/74 (16.2)	13/63 (20.6)	4/84 (4.8)	9/116 (7.8)	<.01
PCR (blood)	4/70 (5.7)	1/55 (1.8)	4/140 (2.9)	2/140 (1.4)	.35
PCR (biopsy specimen)	5/70 (7.1)	9/82 (11.0)	2/188 (1.1)	1/198 (0.5)	<.01
Real-time qPCR (biopsy specimen)	7/202(3.4)	11/144 (7.6)	2/247 (0.8)	12/282 (4.3)	<.01
In situ PCR (biopsy specimen)	0/202(0.0)	1/148 (0.7)	1/251 (0.4)	3/288 (1.0)	.53
Liquid culture (blood)	…	1/31 (3.2)	0/27 (0.0)	2/50 (4.0)	.80
Liquid culture (biopsy specimen)	9/111 (8.1)	8/138 (5.8)	6/232 (2.6)	14/263 (5.3)	.12
Solid culture (biopsy specimen)	…	5/50 (10.0)	0/78 (0.0)	4/97 (4.1)	.02
IHC	2/202 (1)	2/148 (1.3)	2/251 (0.8)	6/288 (2)	.58

Abbreviations: CD, Crohn's disease; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; ITB, intestinal tuberculosis; PCR, polymerase chain reaction; qPCR, quantitative PCR; UC, ulcerative colitis.

Detection of MAP Using Molecular Methods

On PCR analysis of IS900-specific sequence in peripheral blood samples, 1.8% of patients with CD (1 of 55) was positive for MAP, compared with 5.7% of healthy controls (4 of 70), 1.4% of patients with ITB (2 of 140), and 2.9% of patients with UC (4 of 140). On PCR analysis of IS900-specific sequence in biopsy samples, 11% of patients with CD (9 of 82) were positive, compared to 7.1% (5 of 70), 0.5% (1 of 198), and 1.1% (2 of 188) of healthy controls, patients with ITB, and patients with UC, respectively. With real-time qPCR analysis of biopsy

samples, there were significant differences ($P < .01$) among all groups, with the highest positivity rate observed in patients with CD (7.6% [11 of 144]). However, with in situ PCR of biopsy samples, only 1 sample was positive for MAP (Table 3).

Detection of MAP Using Microbial Cultures

With liquid cultures of the biopsy samples, MAP was detected in 5.8% of CD samples (8 of 138) compared to 8.1% (9 of 111), 5.3% (14 of 263), and 2.6% (6 of 232) for non-IBD controls, patients with ITB, and patients with UC, respectively. However, these differences were not statistically significant ($P = .12$). With solid culture of biopsy samples, MAP was detected in 10% of patients with CD (5 of 50), compared to 4.1% (4 of 97) for ITB and 0% (0 of 78) for UC ($P = .02$) (Table 3)

Detection of MAP Using IHC

With IHC staining of biopsy samples, MAP was detected in 1.3% (2 of 148) of patients with CD, and there was no statistically significant difference among the all groups ($P = .58$) (Table 3).

DISCUSSION

In this large, multicenter, nationwide study conducted across various regions of India, we observed that MAP was more commonly detected in patients with CD than in healthy controls as well as patients with UC and ITB, based on findings from serological assays, molecular techniques, microbial culture, and IHC.

The role of MAP in the pathogenesis of CD has been debated for many decades. MAP is a known causative agent of Johne disease in cattle, and, due to the morphological and histological similarities between Johne disease and CD, a causal relationship between MAP and CD has been hypothesized [7]. However, high-quality data supporting this hypothesis are still lacking. Although culture isolation remains the reference standard for MAP diagnosis, the fastidious and slow-growing nature of the organism makes this challenging. In addition, the presence of acid-fast MAP bacilli in patients with CD is rarely reported because MAP can exist in a cell wall–deficient form. In the 1980s, Thayer et al[18] isolated an acid-fast mycobacterium with features similar to MAP from 2 patients with CD. Given these challenges, surrogate markers such as PCR, IHC, and serological detection of antibodies are often used to identify exposure to MAP.

A meta-analysis by Abubakar et al [19], which included 47 observational studies, showed that MAP detected by PCR or in situ hybridization techniques was more frequently associated with CD than with non-IBD controls and patients with UC. However, many studies in this meta-analysis were limited by small sample sizes and lack of blinding. Another meta-analysis by Feller et al [20], examining 28 case-control trials, reported pooled odds ratios of 7.01 (95% confidence interval, 3.95–12.4) for PCR-based detection in tissue samples and 1.72 (1.02–2.90) for ELISA in serum samples. This meta-analysis has been criticized for excluding studies in which MAP was not detected in any patients with CD or controls [20].

PCR amplification using biopsy samples is highly sensitive for detecting MAP. Many observational studies have reported high MAP prevalence in biopsy specimens; however, false-positives due to laboratory contamination cannot be ruled out. Reported MAP prevalence in biopsy samples ranges from 47% to 92% [21–24]. For example, Sanderson et al [25] detected MAP-specific IS900 DNA in 65% of patients with CD compared with 12.5% of controls and 4.3% of patients with UC, while Bull et al [24] demonstrated a prevalence of 92% in patients with CD compared with 26% in non-IBD controls.

Serological evaluation of antibodies against MAP-specific antigens such as p35 or p36 suffers from a lack of specificity and may instead reflect intestinal epithelial barrier dysfunction and subsequent bacterial translocation. A meta-analysis including 13 studies found that 10 reported higher seroprevalence against MAP antigens in patients with CD compared with controls, with a pooled odds ratio of 1.72 (95% confidence interval, 1.02–2.90) [20]. However, Brunello et al [26] found no significant differences in immunoglobulin G antibody titers against MAP protoplasmic antigen between patients with CD (3.7%) and those with UC (5%), and Bernstein et al [27] reported similar seropositivity rates for anti–purified protoplasmic antigen among patients CD (37.8%), patients with UC (34.7%), and healthy controls (33.6%). In our study, the seroprevalence of MAP antibodies in patients with CD was 20.6%, which may reflect impaired intestinal barrier function with increased bacterial translocation. This is supported by elevated antibody titers against other bacteria such as *E. coli*, aerobes, anaerobes, and enteric pathogens in CD.

PCR offers advantages over bacterial cultures by being faster and more sensitive than IHC and in situ hybridization; however, conventional PCR requires nucleic acid extraction and tissue destruction, which precludes correlation with histological features. In situ PCR allows amplification within intact cells, combining sensitivity with spatial localization of specific DNA in tissues, although its sensitivity may be lower than that of conventional PCR [28]. In our study, MAP prevalence assessed by in situ PCR (0.7%) was lower than that detected by qPCR (7.6%) or ELISA (20.6%). Our findings highlight variability among different detection methods. Few patients were positive for MAP by IHC staining, likely due to its low sensitivity, especially when the bacterial burden is low, and because IHC often fails to detect intracellular MAP antigen. Positivity rates were higher in biopsy samples than in blood samples, with PCR and real-time qPCR of biopsy samples showing better detection rates than in situ PCR.

The presence of MAP DNA may not indicate viable bacteria but could reflect residual DNA from environmental exposure and immune responses. Frequent detection of MAP DNA in healthy controls is likely due to its widespread environmental distribution, fecal-oral transmission, and resistance to pasteurization. Singh et al [29] conducted the first large-scale screening of the Indian population, reporting a 34% ELISA positivity rate in 23 196 individuals and an 8.4% IS900 PCR positivity rate in 3093 blood samples.

When compared with herd-level MAP prevalence data, our findings highlight the contrast between widespread environmental exposure and relatively low detection rates in humans. Multiple epidemiological studies show that MAP is highly prevalent in cattle herds; often 20%–60% of herds test positive, depending on the region [30], and even in settings with low animal-level seropositivity (1%–5%), herd-level positivity remains high [31, 32]. In contrast, in our cohort, positivity across culture and molecular methods was generally <10%, with only serology showing a modestly higher signal in patients with CD (20.6%) compared with controls. This discrepancy suggests that although environmental exposure to MAP is frequent, human detection rates are far lower, supporting the notion that MAP may act as one of several contributing factors rather than a ubiquitous agent in CD pathogenesis. It also underscores the complexity of translating high environmental prevalence into disease-specific associations in humans, particularly in regions where both tuberculosis and MAP are endemic.

Despite advances in understanding IBD pathogenesis and the development of biologics and small molecules, therapeutic progress appears to have plateaued [33]. Therefore, elucidating the pathogenesis of CD and identifying new therapeutic targets remain crucial. The pathogenic role of MAP in CD has been debated extensively; unique features of MAP (fastidious culture requirements, extremely slow growth, phenotypic variability, and intracellular survival) and its culture requirements have hindered research. MAP is hypothesized to contribute to CD development through mechanisms such as disruption of intestinal epithelial integrity, triggering gut immune responses, and causing dysbiosis [34]. Future research exploring MAP's pathogenic role could facilitate the development of novel therapies, such as newer combination antimicrobials [35], microbiome manipulation strategies like fecal microbial transplantation, vaccines [10], bacteriophages [11], and host-directed therapies [12].

Our study has several strengths. It was conducted across diverse centers covering a wide geographic area in India and, to the best of our knowledge, includes the largest sample size to date, incorporating both active disease controls and apparently healthy controls. Since MAP detection varies with sample type and detection method, we analyzed both peripheral blood and colonic tissue samples using serological, molecular, and microbiological culture techniques. However, we also acknowledge limitations, such as the unavailability of culture samples from some patients due to limited culture facilities at some centers, and we prioritized biopsy specimens for PCR. In addition, biopsy samples from healthy controls were taken from the sigmoid colon, while those from patients with CD were obtained from the right colon; the implications of this difference remain unclear. We did not specifically exclude patients with a history of earlier antituberculosis treatment for tuberculosis. which may have had an impact on MAP detection. However, MAP differs from *Mycobacterium tuberculosis* in its biology and antibiotic susceptibility, and standard tuberculosis regimens do not reliably eradicate MAP. Therefore, prior tuberculosis treatment cannot be considered equivalent to MAP-targeted therapy.

In conclusion, there is an increased association between MAP and CD as detected by molecular and culture methods, compared with findings in healthy controls and patients with ITB or UC. Future studies should further investigate the potential causal role of MAP in CD and explore therapeutic strategies targeting MAP. Ultimately, a deeper understanding of MAP's involvement could pave the way for novel treatment strategies, including antimicrobial therapies, microbiome modulation, and vaccination approaches, thereby improving outcomes for patients with CD.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Financial support. This work was supported by the Indian Council of Medical Research (grant 5/8/5/28/TF/2013/ECD-I).

Potential conflicts of interest. S. K. V. received a consulting fee from Alimentiv. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. Lancet **2012**; 380:1606–19.
2. Chen W, Li D, Paulus B, Wilson I, Chadwick VS. Detection of *Listeria monocytogenes* by polymerase chain reaction in intestinal mucosal biopsies from patients with inflammatory bowel disease and controls. J Gastroenterol Hepatol **2000**; 15:1145–50.
3. Parent K, Mitchell PD. Bacterial variants: etiologic agent in Crohn's disease? Gastroenterology **1976**; 71:365–8.
4. Müller S, Arni S, Varga L, et al. Serological and DNA-based evaluation of *Chlamydia pneumoniae* infection in inflammatory bowel disease. Eur J Gastroenterol Hepatol **2006**; 18:889–94.
5. Nielsen SS, Toft N. A review of prevalences of paratuberculosis in farmed animals in Europe. Prev Vet Med **2009**; 88:1–14.
6. Lombard JE, Gardner IA, Jafarzadeh SR, et al. Herd-level prevalence of *Mycobacterium avium* subsp. *paratuberculosis* infection in United States dairy herds in 2007. Prev Vet Med **2013**; 108:234–8.
7. Dalziel TK. Chronic interstitial enteritis. Br Med J **1913**; 2:1068–70.

8. Millar D, Ford J, Sanderson J, et al. IS900 PCR to detect *Mycobacterium paratuberculosis* in retail supplies of whole pasteurized cows' milk in England and Wales. Appl Environ Microbiol **1996**; 62:3446–52.
9. Graham DY, Naser SA, Borody T, et al. Randomized, double-blind, placebo-controlled study ofanti-mycobacterial therapy (RHB-104) in active Crohn's disease. Antibiotics **2024**; 13:694. https://doi.org/10.3390/antibiotics13080694
10. Johnston C, Coffey A, O' Mahony J, Sleator R. Development of a novel oral vaccine against *Mycobacterium avium paratuberculosis* and Johne disease. Bioeng Bugs **2010**; 1:155–63.
11. Golla AC, Chaumontet J, Vande Voorde R, Danelishvili L. Discovery of *Mycobacterium avium* subsp. *paratuberculosis* lytic phages with extensive host range across rapid- and slow-growing pathogenic mycobacterial species. Antibiotics **2024**; 13:1009.
12. Crilly NP, Ayeh SK, Karakousis PC. The new frontier of host-directed therapies for *Mycobacterium avium* complex. Front Immunol **2021**; 11:623119.
13. Mameli G, Cocco E, Frau J, Marrosu MG, Sechi LA. Epstein Barr virus and *Mycobacterium avium* subsp. *paratuberculosis* peptides are recognized in sera and cerebrospinal fluid of MS patients. Sci Rep **2016**; 6:22401.
14. Quayle AJ, Wilson KB, Li SG, et al. Peptide recognition, T cell receptor usage and HLA restriction elements of human heat-shock protein (hsp) 60 and mycobacterial 65-kDa hsp-reactive T cell clones from rheumatoid synovial fluid. Eur J Immunol **1992**; 22:1315–22.
15. Maaser C, Sturm A, Vavricka SR, et al. ECCO-ESGAR guideline for diagnostic assessment in IBD part 1: initial diagnosis, monitoring of known IBD, detection of complications. J Crohns Colitis **2019**; 13:144–64K.
16. Kedia S, Das P, Madhusudhan KS, et al. Differentiating Crohn's disease from intestinal tuberculosis. World J Gastroenterol **2019**; 25:418–32.
17. Khan IA, Nayak B, Markandey M, et al. Differential prevalence of pathobionts and host gene polymorphisms in chronic inflammatory intestinal diseases: Crohn's disease and intestinal tuberculosis. PLoS One **2021**; 16:e0256098.
18. Thayer WR, Coutu JA, Chiodini RJ, Van Kruiningen HJ, Merkal RS. Possible role of mycobacteria in inflammatory bowel disease. Digest Dis Sci **1984**; 29:1080–5.
19. Abubakar I, Myhill D, Aliyu SH, Hunter PR. Detection of *Mycobacterium avium* subspecies *paratuberculosis* from patients with Crohn's disease using nucleic acid-based techniques: a systematic review and meta-analysis. Inflamm Bowel Dis **2008**; 14:401–10.
20. Feller M, Huwiler K, Stephan R, et al. *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease: a systematic review and meta-analysis. Lancet Infect Dis **2007**; 7:607–13.
21. Autschbach F, Eisold S, Hinz U, et al. High prevalence of *Mycobacterium avium* subspecies *paratuberculosis* IS900 DNA in gut tissues from individuals with Crohn's disease. Gut **2005**; 54:944–9.
22. Mintz MJ, Lukin DJ. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and Crohn's disease: the debate continues. Transl Gastroenterol Hepatol **2023**; 8:28.
23. Zarei-Kordshouli F, Geramizadeh B, Khodakaram-Tafti A. Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* IS 900 DNA in biopsy tissues from patients with Crohn's disease: histopathological and molecular comparison with Johne's disease in Fars province of Iran. BMC Infect Dis **2019**; 19:23.
24. Bull TJ, McMinn EJ, Sidi-Boumedine K, et al. Detection and verification of *Mycobacterium avium* subsp. *paratuberculosis* in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. J Clin Microbiol **2003**; 41:2915–23.
25. Sanderson JD, Moss MT, Tizard ML, Hermon-Taylor J, J. Mycobacterium paratuberculosis DNA in Crohn's disease tissue. Gut **1992**; 33:890–6. doi:10.1136/gut.33.7.890
26. Brunello F, Pera A, Martini S, et al. Antibodies to *Mycobacterium paratuberculosis* in patients with Crohn's disease. Dig Dis Sci **1991**; 36:1741–5.
27. Bernstein CN, Blanchard JF, Rawsthorne P, Collins MT. Population-based case control study of seroprevalence of *Mycobacterium paratuberculosis* in patients with Crohn's disease and ulcerative colitis. J Clin Microbiol **2004**; 42:1129–35.
28. Bagasra O. Protocols for the in situ PCR-amplification and detection of mRNA and DNA sequences. Nat Protoc **2007**; 2:2782–95.
29. Singh SV, Kumar N, Sohal JS, et al. First mass screening of the human population to estimate the bio-load of *Mycobacterium avium* subspecies *paratuberculosis* in North India. J Public Health Epidemiol **2014**; 6:20–9.
30. Corbett CS, Naqvi SA, Bauman CA, et al. Prevalence of *Mycobacterium avium* ssp. *paratuberculosis* infections in Canadian dairy herds. J Dairy Sci **2018**; 101:11218–28.
31. Johnson P, Marfleet T, Waldner C, Parker S, Campbell J. Seroprevalence of *Mycobacterium avium* spp. *paratuberculosis* in cow-calf herds located in the prairie provinces of Canada. Can Vet J **2022**; 63:1247–51.
32. Liu X, Li J, Yang X, Wang D, Wang J, Wu J. The seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* in dairy cattle in Xinjiang, Northwest China. Ir Vet J **2017**; 70:1.
33. Raine T, Danese S. Breaking through the therapeutic ceiling: what will it take? Gastroenterology **2022**; 162:1507–11.
34. Agrawal G, Borody TJ, Aitken JM. Mapping Crohn's disease pathogenesis with *Mycobacterium paratuberculosis*: a hijacking by a stealth pathogen. Dig Dis Sci **2024**; 69:2289–303.
35. Alcedo KP, Thanigachalam S, Naser SA. RHB-104 triple antibiotics combination in culture is bactericidal and should be effective for treatment of Crohn's disease associated with *Mycobacterium paratuberculosis*. Gut Pathog **2016**; 8:32.