

# Enteropathogenic Viruses: Triggers for Exacerbation in IBD? A Prospective Cohort Study Using Real-time Quantitative Polymerase Chain Reaction.

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# Enteropathogenic Viruses: Triggers for Exacerbation in IBD? A Prospective Cohort Study Using Real-time Quantitative Polymerase Chain Reaction

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**Background:** While the role of bacteria as an etiological factor triggering relapse in inflammatory bowel disease (IBD) has been studied extensively, little is known of the role of enteric viruses. We aimed to prospectively study the prevalence and risk factors for common enteropathogenic viruses in IBD patients in relation to disease activity.

**Methods:** IBD patients visiting the outpatient clinic of the Maastricht University Medical Center were included in a prospective cohort with a follow-up of 1 year. Every 3 months and during relapses, fecal samples, demographic, and clinical data were collected and disease activity was scored. A fecal sample from patients at baseline (Crohn's disease [CD]  $n = 170$ , ulcerative colitis [UC]  $n = 116$ ) and an additional sample from a subgroup with changing disease activity during follow-up (CD  $n = 57$ , UC  $n = 31$ ) were analyzed for the presence of rotavirus, norovirus GI and GII, human astrovirus, and adenovirus using quantitative polymerase chain reaction (qPCR).

**Results:** Overall viral pathogen detection, defined by the detection of at least one of the studied viruses, at baseline was 5.2% and differed neither between CD (6.5%) or UC patients (3.4%) ( $P = 0.20$ ), nor between active disease (4.7%) and remission (5.5%) ( $P = 0.79$ ). Within the subgroup of patients with changing disease activity no association was found between overall viral pathogen detection and disease activity ( $P = 0.39$ ). Using multivariate logistic regression, age, gender, disease subtype, disease activity, medication, and season of sampling were not associated with overall viral pathogen detection.

**Conclusions:** Enteropathogenic viruses are not frequently observed in a consecutive cohort of IBD patients and are not a common trigger for active disease in daily clinical practice.

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**Key Words:** inflammatory bowel disease, virus, etiology, enteric pathogen, real-time polymerase chain reaction

Our knowledge of the role of the intestinal microbiota in health and disease is rapidly increasing.<sup>1–3</sup> This has resulted in more detailed insight of microbial involvement in the pathogenesis of inflammatory bowel diseases (IBD). This is based on the presence of high mucosal bacterial numbers<sup>4</sup> and findings that diversion of the fecal stream<sup>5</sup> and antibiotic therapy<sup>6</sup> resulted in decreased disease activity. Murine colitis models further support the importance of the microbiota in the induction and perpetuation of intestinal inflammation.<sup>7</sup>

Although a dysbalance of the (fecal and mucosal) commensal microbiota has been demonstrated in patients with IBD,<sup>4,8,9</sup> and differences in microbiota in relation to disease subtype is described,<sup>4</sup> so far no specific bacterial species or groups have been identified to be causative or associated with an exacerbation. In addition to a putative role of commensal bacteria, gastrointestinal pathogens have been suggested to induce intestinal symptoms and/or inflammation. Associations have been reported between acute gastroenteritis and onset or exacerbations of IBD.<sup>10–12</sup> Considering a potential role for microbial pathogens in inducing flares in IBD, one should take into account that gastrointestinal infections are caused most frequently by enteric viruses such as rotavirus and norovirus instead of bacteria.<sup>13,14</sup> Recent experiments in animals do point to a role of enteric viruses in the progression of colitis. It was shown that murine norovirus worsened colitis induced by *Helicobacter bilis*.<sup>15</sup> In another mouse model, murine norovirus induced Paneth cell dysfunction in genetically susceptible mice.<sup>16,17</sup> In human studies, cytopathic effects have been observed in cell monolayers induced by administration of homogenates of tissue obtained from Crohn's disease (CD)

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patients. These cytopathic effects have been attributed to a virus.<sup>18,19</sup> However, these observations were not specific, since similar effects have been obtained with isolates of tissue retrieved from patients with other chronic intestinal disorders.<sup>20</sup> Other investigators have not been able to confirm these findings<sup>21,22</sup> and sero-logical studies did not find an association between sero-conversion of antibodies against rotavirus, Norwalk virus (currently named norovirus), or adenovirus and clinical relapse or IBD subtype.<sup>23,24</sup> No association was observed between the presence of antibodies against adenovirus and regional enteritis in ulcerative colitis (UC)<sup>22,24,25</sup> or antibody prevalence and titer to rotavirus or Norwalk virus in CD compared to matched controls.<sup>26</sup>

A recent retrospective study evaluating noroviruses in pediatric IBD patients could not specify a role for norovirus in initiation or exacerbation of IBD.<sup>27</sup> Unfortunately, the data were based on very small numbers (i.e., nine patients) and a traditional diagnostic technique (i.e., enzyme immunoassay) was used. Although up to now studies in humans have not been able to demonstrate a potential role for enteric viruses in IBD, these interpretations of data were hampered by shortcomings in the diagnostic technique or in sample size. Prospective studies using state of the art molecular techniques in larger sets of adult IBD patients are currently lacking. Such state of the art diagnostic techniques have proved to be very accurate and concordant in viral detection rate when compared to traditionally used methods.<sup>28,29</sup> Real-time quantitative polymerase chain reaction (qPCR) is emerging as a method for detection of enteric viruses in feces.<sup>30–32</sup>

Considering the possible limitations of the previous human studies, and the potential role of enteric viruses in the progression of colitis in experimental mice models, further studies are warranted to investigate whether enteric viruses are associated with development of active disease in IBD. This holds especially true for patients while on immunosuppressive agents. Therefore, we aimed to investigate the prevalence of common enteric viruses in relation to disease activity in a consecutive prospective cohort of IBD patients, and second, to determine risk factors for enteric viral infections within the same population.

## MATERIALS AND METHODS

### Patients

Between August 2009 and November 2010, consecutive IBD patients visiting the outpatient IBD clinic of our combined secondary and tertiary hospital (Maastricht University Medical Centre) were asked to participate and upon agreement were included in a prospective cohort study with 1-year follow-up after inclusion. The diagnosis of CD or UC was based on endo-scopical, histological, and/or radiological findings. Patients visited the hospital every 3 months and at time of relapse, during which

fecal samples, demographic, and clinical data were collected using standardized sampling and registration protocols.

Data of concomitant use of medication, smoking status (current smoker, quit smoking within or over 6 months, never smoked), body mass index (BMI), disease duration since diagnosis, and disease phenotype were obtained using the computer-based medical registration databases. Disease activity was scored by the Harvey–Bradshaw index for CD patients,<sup>33</sup> (remission defined as score <5), the Simple Clinical Colitis Activity Index for UC patients<sup>34</sup> (remission defined as score <3), and the Pouchitis Disease Activity Index for UC patients with a pouch<sup>35</sup> (remission defined as solely clinical score ≤2, or solely endoscopic score ≤1, or total score, combination of clinical and endoscopic score ≤4).

Patients were provided with a small plastic container (Fisher Scientific, Landsmeer, The Netherlands) and instructed to collect a fecal sample on the evening before or the morning of each visit and to store the sample at 4°C. Upon arrival at the laboratory, undiluted feces was frozen at –80°C within 24 hours after defecation.

Fecal samples from the inclusion visit of all patients were used for further analyses in the present study (i.e., baseline cohort). Furthermore, from a subgroup of patients with changing disease activity during follow-up, a second sample was evaluated (i.e., follow-up cohort).

### Viral Diagnostics

For the present study, the most common enteric viruses, i.e., rotavirus, norovirus G I and II, human astrovirus, and adenovirus<sup>13,14,36</sup> were analyzed. Before RNA and DNA extraction, all samples were spiked with murine cytomegalovirus (mCMV) RNA, which was used as an extraction and amplification control. The procedure for DNA and RNA isolation has been described in detail elsewhere.<sup>28</sup>

Briefly, feces was homogenized with the MagNA Lyser (Roche Diagnostics, Almere, The Netherlands). DNA and RNA were isolated using the MagNA Pure total NA extraction kit by the MagNA Pure LC system (Roche Diagnostics).

The detection of the viruses under study was conducted by real-time PCR using the primers and probes described in Table 1. For the detection of RNA viruses, this was preceded by a reverse transcription (RT) step using TaqMan reverse transcriptase reagents by utilizing random hexamers (Applied Biosystems, Foster City, CA) and consisted of 10 minutes at 25°C, 30 minutes at 48°C, 5 minutes at 95°C, and finally a cooling phase down to 4°C.

The real-time PCR mix for each reaction consisted of 10 μL isolated DNA, 900 nM of both the forward and reverse primers, 300 nM probe, 12.5 μL Absolute quantitative PCR mix (Abgene, Epsom, UK), and additional NASBA water to reach a final reaction volume of 25 μL. The PCR protocol consisted of 15 minutes at 95°C, followed by 42 cycles of 15 seconds at 95°C and 1 minute at 60°C and was reported in more detail previously.<sup>28</sup> The quality of the assays was ensured by positive and negative controls as well as a test on amplification inhibition in

**TABLE 1.** Sequences of Primers and Probes Used for the Detection of Enteric Viruses

Target Organism	Primer/Probe	Sequence (5'–3')	Reference
Adenovirus	Forward primer	GCCCCAGTGGTCTTACATGCACATC	31
	Reverse primer	GCCACGGTGGGGTTTCTAAACTT	31
	Probe	FAM-TGCACCAGACCCGGGCTCAGGTACTCCGA-BQ1	31
Human astrovirus	Forward primer	TCTYATAGACCGYATTATTGG	63
	Reverse primer	TCAAATTCTACATCATCACCAA	63
	Probe	FAM-CCCCADCCATCATCATCTTCATCA-TAMRA	63
Norovirus GI	Forward primer	CGYTGGATGCGNTTYCATGA	63
	Reverse primer	CCTTAGACGCCATCATCATTTAC	63
	Probe	FAM-TYGCGRCTCCTGTCCA-MGBNFQ	63
Norovirus GII	Forward primer	CARGARBCNATGTTYAGRTGGATGAG	63
	Reverse primer	TCGACGCCATCTTCATTCACA	63
	Probe	VIC-AGATYGCATCSCCCTC-MGBNFQ	63
Rotavirus	Forward primer	ACCATCTTCACGTAACCCTC 15	64
		ACCATCTACACATGACCCTC (2 <sup>nd</sup> forward primer)	
	Reverse primer	CACATAACGCCCTATAGCC	64
	Probe	FAM-ATGAGCACAATAGTAAAAGCTAACTGTTCAA-BQ1	64

FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine; BQ1, black hole quencher 1; MGBNFQ, minor groove binding nonfluorescent quencher; VIC, fluorescent label (Applied Biosystems).

each sample by an external amplification control (mCMV). Artificial RNA controls were constructed using pGEM-3Z vectors containing T7 RNA polymerase promoters flanking the multiple cloning region (Promega, Leiden, The Netherlands) into which the respective amplicons were cloned. Using T7 RNA polymerase, RNA constructs containing the amplicons were generated and used as positive RNA controls.

Samples were considered positive when a virus was detected, irrespective of the Ct value at which the virus was detected. The overall viral pathogen detection was defined by the detection of at least one of the studied viruses.

### Statistical Analysis

The chi-square test was used to compare dichotomous variables between subgroups of patients. In case of too-small numbers, Fisher's exact test was performed.

Multivariate logistic regression analyses were performed for the identification of risk factors (disease subtype [CD or UC], disease activity [active disease or remission], gender, immunosuppressive therapy [corticosteroids, methotrexate and/ or thiopurines], anti-tumor necrosis factor alpha [TNF- $\alpha$ ] therapy, age, and season of sample collection) for overall viral pathogen detection. Classification of sample collection per season was based on the date of fecal sample collection.

McNemar's test was used to examine the association between disease activity and overall viral pathogenic detection within the subgroup of subjects of whom both a fecal sample during active disease and remission were available (follow-up cohort).

Statistical analyses were performed using SPSS v. 18.0 (Chicago, IL). Statistical significance was defined as  $P < 0.05$ .

### Ethical Considerations

The study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre, Maastricht, and written informed consent was obtained from all patients.

## RESULTS

### Patients

A total of 323 patients were included in the IBD study cohort. A baseline fecal sample was available from 286 patients (baseline cohort). Baseline characteristics of these patients are shown in Table 2. Mean age at baseline was 46.2 years (SD 15.2), with mean disease duration of 12.4 years (SD 9.9). CD and UC were diagnosed in 59% and 41% (including 15% with an ileal-anal pouch) of the study population, respectively. Active disease was present in 85 patients (29.7%), and was comparable in CD (33.5%) versus UC (25.0%) patients. During the 1-year follow-up period, disease activity changed over time in 94 patients, of whom 88 (follow-up cohort) provided fecal samples during both remission and relapse of disease.

### Enteric Viruses

#### Baseline

The overall viral pathogen detection at baseline was 5.2% in all IBD patients and did not differ significantly

**TABLE 2.** Characteristics of IBD Patients in the Baseline Cohort ( $n=286$ )

	CD $N=170$ $N$ (%)	UC $N=116$ $N$ (%)
Active disease	57 (33.5)	29 (25.0)
Remission	113 (66.5)	87 (75.0)
Male	60 (35.3)	62 (53.4)
Mean age in yrs (SD)	44.0 (15.0)	49.4 (14.9)
Disease duration in yrs (SD)	12.2 (10.7)	12.5 (8.6)
Disease phenotype		
Nonpenetrating, nonstricturing	118 (69.4)	
Stricturing	29 (17.1)	
Penetrating	16 (9.4)	
Stricturing and penetrating	7 (4.1)	
Disease location		
Colon	38 (22.4)	
Small bowel only	48 (28.3)	
Ileocolonic	84 (49.4)	
Medication use	144 (84.7)	105 (90.5)
Immunosuppressive therapy	124 (72.9)	53 (45.7)
Thiopurin	60 (35.3)	30 (25.9)
Azathioprin	35 (20.6)	14 (12.1)
Mercaptopurin	25 (14.7)	16 (13.8)
Methotrexate	9 (5.3)	4 (3.4)
Anti-TNF alpha therapy	75 (44.1)	24 (20.7)
Infliximab	55 (32.4)	24 (20.7)
Adalimumab	19 (11.2)	
Other	1 (0.6)	
Corticosteroids	25 (14.7)	10 (8.6)
Prednisone	4 (2.4)	7 (6.1)
Budesonide	21 (12.4)	4 (3.4)
Aminosalicylates		
Systemically	33 (19.4)	61 (52.6)
Locally	2 (1.2)	18 (15.5)
Immunosuppressive agents therapy		
none	46 (27.1)	63 (54.3)
1	86 (50.6)	39 (33.6)
$\geq 2$	38 (22.4)	14 (12.1)

CD, Crohn's disease; UC, ulcerative colitis.

between CD (6.5%) and UC (3.4%) patients ( $P = 0.20$ ) (Fig. 1). Adenoviruses were detected more frequently (3.8% in IBD patients) compared to the other viruses, without any preference for disease subtype ( $P = 0.52$ ). Rotavirus and norovirus GI were detected in 1.0% and 0.3%, respectively. Human astrovirus and norovirus GII were not detected in any of the fecal samples at baseline. No coinfections were observed. Prevalences of the single viruses were not different between UC and CD.

The prevalence of the single viruses and overall viral pathogen detection in CD, UC, and IBD patients in relation to disease activity is shown in Table 3. Comparing active patients with those in remission, no significant differences were found for overall viral pathogen detection ( $P = 0.79$ ), nor for single enteric viruses.

As no differences for overall viral pathogen detection were found between CD and UC, the multivariate analysis was performed with the total IBD population, including disease subtype as covariate (Table 4). No statistically significant associations were found between overall viral pathogen detection and disease subtype, disease activity, gender, age, immunosuppressive therapy, anti-TNF- $\alpha$  therapy, or season of sample collection. The timing of sample collection of the baseline cohort was equally distributed over the seasons (data not shown).

### Follow-up Cohort

From 88 subjects subsequent samples were analyzed during follow-up. From 39 patients (27 CD and 12 UC) disease activity changed from inactive toward active disease and from 49 patients (30 CD and 19 UC) from active toward inactive disease during follow-up. The mean time between subsequent samples in both groups was 110 (SD  $\pm 71$ ) and 153 (SD  $\pm 87$ ) days, respectively.

In this follow-up cohort where alterations in disease activity occurred during follow-up, overall viral pathogen detection did not differ between active disease (4.0%) and remission (7.7%) (McNemar's test for paired proportions,  $P = 0.39$ ). Within the group of active disease at baseline, two CD patients tested positive for an enteric virus, whereas during follow-up in three CD and three UC patients a virus was detected in the fecal sample taken during remission. Only one CD patient had two consecutive positive fecal samples for rotavirus: at baseline (i.e., active disease) as well as during subsequent remission after a follow-up of 104 days. Within the group of remission in which two CD patients and one UC patients tested positive for an enteric virus at baseline, during follow-up an enteric virus was detected in the fecal sample taken during active disease in two CD patients and one UC patient.

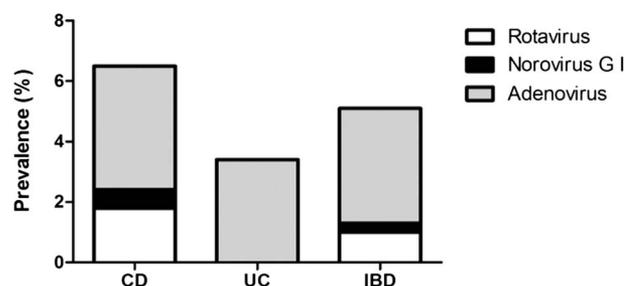


FIGURE 1. Prevalence and distribution of the detected separate viruses in CD, UC, and all IBD patients at baseline.

**TABLE 3.** Prevalences (%) of Enteric Viruses and Overall Viral Pathogen Detection in Fecal Samples of the Baseline Cohort

Organism	CD		UC		IBD	
	A N=57 N (%)	R N=113 N (%)	A N=29 N (%)	R N=87 N (%)	AN=86 N (%)	R N=200 N (%)
Rotavirus	2 (3.5)	1 (0.9)	0	0	2 (2.3)	1 (0.5)
Norovirus GI	0	1 (0.9)	0	0	0	1 (0.5)
Norovirus GII	0	0	0	0	0	0
Human astrovirus	0	0	0	0	0	0
Adenovirus	2 (3.5)	5 (4.4)	0	4 (4.6)	2 (2.3)	9 (4.5)
Overall viral pathogen detection	4 (7.0)	7 (6.2)	0	4 (4.6)	4 (4.7)	11 (5.5)

Presented for Crohn’s disease (CD), ulcerative colitis (UC), and all IBD patients during active disease (A) and remission (R).

**DISCUSSION**

To our knowledge, this is the first study investigating the role of common enteric viruses in a prospective cohort of adult IBD outpatients by real-time qPCR. We report a low overall prevalence of enteric viruses in IBD patients. No association was observed between overall viral pathogen detection and IBD subtype, disease activity, or use of immunosuppressive therapy.

The overall viral pathogen detection rate at baseline in IBD patients is low (5.2%) and did not differ significantly between CD and UC patients. For comparison, data on enteric virus prevalences in a community setting using molecular diagnostic techniques on fecal samples are limited. A study in a population-based Dutch cohort of asymptomatic subjects reported a prevalence of 9.1%.<sup>13</sup> This prevalence of enteric viruses is higher than we observed in IBD and may at least partly be explained by the fact that this population study also tested for sapovirus. Moreover, 54% of the subjects were children up to the age of 11 years, which are known to be more susceptible to enteric viruses<sup>17</sup> and the prevalence and fecal shedding of enteric viruses in children is reported to be higher.<sup>37,38</sup> Another study investigated the prevalence of rotavirus and adenovirus in asymptomatic subjects in a community setting and reported viral pathogen detection in 0.1%.<sup>39</sup> It should be noted that these findings were based on latex agglutination tests. When molecular techniques are used, the prevalences of adenovirus, rotavirus, norovirus, sapovirus, and human astrovirus in controls is generally higher.<sup>29</sup> Despite considerable differences in the reported prevalence of enteric viruses related to diagnostic techniques used and the populations, the low overall prevalence observed in IBD patients is in agreement with data obtained in the general population.

In our cohort, the viral detection rate was highest for adenoviruses (3.8%). This is in line with observations in patients with acute gastroenteritis<sup>13</sup> but is higher in comparison to healthy controls<sup>40</sup> and to the prevalence of enteric adenovirus (Ead) in gastroenteritis patients.<sup>41</sup> However, we cannot

conclude that our reported prevalence can be fully accounted for by enteric adenoviruses, since we did not use primers specific for serotype 40 and 41 (Ead). Human astrovirus was not detected in our study, in line with the low prevalence in healthy

**TABLE 4.** Multivariate Analysis for the Identification of Risk Factors for Overall Viral Pathogen Detection in the Baseline Cohort (n=286)

Variables	OR	95% CI	P-value
Disease subtype			
CD	1	—	
UC	0.38	0.11–1.36	0.14
Disease activity			
Remission	1	—	
Active disease	1.05	0.30–3.60	0.94
Gender			
Male	1	—	
Female	0.45	0.14–1.42	0.17
Age (years)	0.99	0.95–1.02	0.45
Immunosuppressive therapy *			
No	1	—	
Yes	1.76	0.58–.35	0.32
Anti-TNF-alpha therapy			
No	—	—	
Yes	0.26	0.05–1.30	0.10
Season			
Summer	1	—	
Autumn	0.34	0.04–2.88	0.32
Winter	0.94	0.16–5.49	0.95
Spring	1.59	0.28–8.97	0.60

CD, Crohn’s disease; UC, ulcerative colitis; OR, odds ratio; CI, confidence interval. \*Combination of corticosteroids, methotrexate, and/or thiopurines.

controls,<sup>13</sup> but also in acute gastroenteritis patients.<sup>41</sup> Noroviruses were anticipated to be observed more frequently. Our data do not confirm this with a low prevalence at baseline. In follow-up we detected noroviruses (both GI and GII) in seven samples. Although the applied real-time PCR method allows for the determination of an exact viral load (in copies or genome equivalents), this was not determined in our study, as factors such as the consistency and water content of the fecal samples influence the viral load. No correction for these factors was included in this study because of the small number of viral-positive samples.

With the application of sensitive molecular methods in a prospective cohort of IBD patients, we found neither an association between disease subtype or disease activity with overall viral pathogen detection nor with the presence of any of the enteropathogenic viruses studied. Although the results of several older studies might be limited due to the use of conventional techniques,<sup>20–26</sup> our findings strongly add evidence to these early reports and assumptions. However, our data are in contrast to those obtained in mice models, which indicate a potential role for enteric viruses in the pathogenesis of IBD.<sup>15–17</sup> The low overall viral pathogen detection rate, as found in our study, however, contradicts the importance of such an association.

By multivariate analysis no risk factors could be identified, which was to be expected based on the small number of positive samples. No seasonal influences regarding sampling were noticed, considering seasonal variability of separate viruses.<sup>41–43</sup> Seasonal variation in flares of IBD has been suggested, although the results are inconsistent.<sup>44–52</sup> Both findings support our results, and emphasize the lack of association between viral pathogens and relapse in IBD.

Following patients consecutively in relation to a change of disease activity over time, especially the transition from inactive toward active disease, permitted us to investigate the role of enteric viruses in triggering relapses of disease. Analysis of overall viral pathogen detection within subjects with changing disease activity showed no relation between viral detection and IBD subtype or disease activity. We identified only one patient in whom two consecutive samples were positive for rotavirus, with decreasing quantity over time (data not shown). In the follow-up cohort the mean time between first and second sample was 129 days and we thereby excluded the presence of chronic viral infection in the studied patients. In healthy adults viral excretion is observed with a maximum of 8 weeks after inoculation,<sup>53</sup> whereas chronic viral infection has been reported in severely immunocompromised patients.

Whether the risk of serious and/or opportunistic infections in patients using immunosuppressive, modulating drugs, or anti-TNF- $\alpha$  agents is increased is currently under debate.<sup>54–56</sup> Our data do not support such an association for enteric viruses in an unselected prospective cohort of IBD patients from a combined secondary and tertiary hospital, thereby representing daily clinical practice.<sup>57–59</sup> As expected, the follow-up cohort received

significantly more often anti-TNF- $\alpha$  therapy ( $P < 0.001$ ) and immunosuppressive agents ( $P < 0.001$ ) (data not shown) compared to those with only one sample analyzed, presumably due to the disease activity and severity of patients from the follow-up cohort.

Even though a significantly higher proportion of patients receiving anti-TNF- $\alpha$  therapy at baseline had active disease, neither disease activity nor use of anti-TNF- $\alpha$  therapy influenced overall viral pathogen detection. It is known that the impact of thiopurine therapy on the host immune function is mainly through inhibition of T lymphocytes,<sup>60</sup> which are important in the prevention of viral infection. Considering the high number of patients at baseline using thiopurine therapy, the likelihood for overall viral pathogen detection in patients using immunosuppressive therapy use was not increased. The question of whether the use of specific medication is accompanied by an increased susceptibility for viral infection should be based on a larger group of IBD patients.

We acknowledge a few limitations of the present study. First, the period of median viral excretion after infection in adults was 4 weeks.<sup>53</sup> We cannot exclude that patients will have had a viral infection before attending the clinic. However, patients experiencing gastrointestinal symptoms, perhaps attributable to an exacerbation, are instructed to contact our clinic for further diagnostics and necessary adaptation of therapy. In addition, patients usually visit the outpatient clinic within an interval of less than 2 weeks after onset of the first symptoms. Second, the duration of relapse in patients with active disease at baseline has not been taken into account. Since we were interested in prevalences of enteric viruses in both active and inactive disease and we furthermore wanted to elucidate the role of viruses in fluctuations in disease activity, the prospective design and analysis within the follow-up cohort will have markedly reduced, if not excluded, this influence.

Within the current study we used a hypothesis-driven approach targeting the by far most common enteropathogenic viruses. Although unlikely, given the already low prevalence of the viruses under study, we cannot rule out the potential influence of other (less common or unknown) viruses with certainty.

In this respect, the application of metagenomics to study the intestinal virome in a more explorative manner may provide additional insights. The implementation of viral metagenomic approaches has already increased our knowledge of viruses inhabiting the human gut, but also has illustrated that the vast majority of viruses identified from human feces are not animal viruses but rather bacterial viruses (phages)<sup>61</sup> or plant viruses<sup>62</sup> derived from contaminated food.

The strength of the current study is that from a large group of adult IBD patients representative for daily clinical practice, clinical data and consecutive fecal samples were available for analyses. Since from a subgroup of patients with alterations in disease activity additional fecal samples were analyzed, insight into the potential role of enteric viruses in triggering active disease

of IBD could be investigated. We used sensitive molecular diagnostic techniques for viral detection.

## CONCLUSION

In this prospective study we report a very low prevalence of enteric viruses in IBD patients visiting the outpatient clinic. No risk factors for viral infection were identified. Our findings indicate that common enteric viral pathogens are not a major trigger for exacerbations in IBD in daily clinical practice.

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