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Short Report

Where have the enteric viruses gone? - Differential effects on frequent causes of infectious diarrhoea by SARS-CoV-2 pandemic lockdown measures

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S U M M A R Y

Background: Measures of distancing, wearing face/medical masks and lockdown introduced in many countries to meet the challenges of the SARS-CoV-2 pandemic have led to gross changes in the epidemiology of important infections. The observation of decline of positive norovirus tests after introduction of lockdown in Germany led us to investigate changes in the detection of major causes of diarrhoea by comparing pre-pandemic quarters (PPQ: 1Q/17 through 1Q/20) since 2017 and pandemic quarters (PQ: 2Q/20 through 1Q/21).
Methods and setting: Bioscientia Laboratory Ingelheim is a large regional clinical pathology laboratory serving > 50 hospitals and > 5000 general practitioners and specialist outpatient practices located in the federal states Hesse, Rhineland-Palatinate and North Rhine-Westphalia, Germany. Antigen detection assays were used for detection of astrovirus, adenovirus, rotavirus, and *Campylobacter* antigen and *Clostridium difficile* Toxin A/B, while norovirus was detected by qualitative RT-PCR.
Findings: The mean positivity-ratios of norovirus, adenovirus and astrovirus assays were 3–20 fold lower in periods PQ (2Q/20 through 1Q/21) compared to PPQ (1Q/17 through 1Q/20) (p<.01). The mean positivity-ratio was lower in PQ compared to PPQ for rotavirus (p=.31), but failed to reach statistical significance, while for campylobacter antigen (p=.91) and *C. difficile* Toxin A/B (p=.17) the mean positivity-ratio was even higher in PQ compared to PPQ.
Conclusions: Apparently, hygienic measures used to contain the SARS-CoV-2 pandemic have differential effects on incidence of diarrhoea viruses as compared to bacterial gastrointestinal agents, particularly *C. difficile*, which may lead to re-evaluate measures implemented against this important cause of nosocomial diarrhoea.

Abbreviations: PPQ, Pre-pandemic quarters 1Q/17 – 1Q/20; PQ, Pandemic quarters 2Q/20 – 1Q/21.

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Introduction

Since declaration of the SARS-CoV-2 pandemic state by the World Health Organisation on 11th March 2020 the pandemic has gone through three main phases in Germany [1]. These included a general lockdown with contact inhibition and closures of shops, schools, theatres and restaurants from week 13/2020, introduction of mandatory face-mask use in many public areas from week 18, another partial lockdown from week 45 (strengthened into a complete lockdown from week 51/2020) which was still ongoing with some opening of shops and schools in the time period investigated [1]. Similar measures of distancing, wearing face/medical masks and lockdown have been introduced in other countries to meet the challenges of the SARS-CoV-2 pandemic. Recently, it was reported that those measures have been successful in preventing infections and epidemics with other respiratory viruses like influenza A in Australia, the US and Europe [2–4].

The laboratory observation that the number of positive norovirus tests has declined dramatically since introduction of the first lockdown in Germany and other countries [5,6] led us to investigate changes in the detection of major causes of diarrhoea in our laboratory when comparing pre-pandemic quarters (PPQ: 1Q/17 through 1Q/20) since 2017 and pandemic quarters (PQ: 2Q/20 through 1Q/21).

Methods

Setting

Bioscientia Laboratory Ingelheim is a large regional clinical pathology laboratory serving > 50 hospitals and > 5000 general practitioners and specialist outpatient practices located in the federal states Hesse, Rhineland-Palatinate and North Rhine-Westphalia, Germany. Tests are done as ordered by the requestor, e.g. there is no restriction to test only outbreak specimens for norovirus, but sporadic and outbreak specimens are tested as requested.

Diagnostic methods

Antigen detection assays were used for detection of astrovirus, adenovirus, and rotavirus (Serazym® Adenovirus, Ref. E-017-A2 Serazym® Astrovirus, Ref. E-045-A2, Serazym® Rotavirus, Ref. E-020-A2, Seramun Diagnostika GmbH, Germany), *Campylobacter* antigen and *Clostridium difficile* Toxin A/B (*Campylobacter* REF 318080, C. *difficile* Toxin A&B Assay REF 318900, DiaSorin LIAISON, Germany), while norovirus was detected by qualitative RT-PCR (RealStar® Norovirus RT-PCR Kit 3.0 Art.-Nr. 053013, Altona, Germany).

Data and data analysis

The number of tests performed per quarter are displayed in Table I and varied for the different agents of diarrhoea from a minimum of N=183 tests per quarter for astrovirus and a maximum of N=8494 tests per quarter for campylobacter. For the same organism there were also some differences in test numbers between quarters in general and between pre-pandemic and pandemic quarters, however, generally these were not greater than twofold.

The study used only anonymised data from routine tests performed in-house and therefore ethical approval was not necessary according to the responsible ethics committee in our region.

Table I
Positivity ratios of assays for agents of diarrhoea in pre-pandemic and pandemic quarters*

Organism / Period	**Pre-pandemic quarters (PPQ)**													**Pandemic quarters (PQ)**			
Positivity-ratio (%)	**1Q/17**	**2Q/17**	**3Q/17**	**4Q/17**	**1Q/18**	**2Q/18**	**3Q/18**	**4Q/18**	**1Q/19**	**2Q/19**	**3Q/19**	**4Q/19**	**1Q/20**	**2Q/20**	**3Q/20**	**4Q/20**	**1Q/21**
Adenovirus	11,9	5,7	3,7	4,2	4,7	3,4	4,2	4,9	4,2	4,0	3,6	1,8	2,9	0,5	2,8	1,8	1,0
Astrovirus	5,5	6,2	6,5	7,5	5,5	7,9	8,2	5,9	7,2	5,9	3,2	1,4	1,3	0,0	0,0	0,0	0,0
C. difficile-Toxin A&B	14,4	16,5	11,7	15,3	16,0	16,8	11,8	13,0	13,1	11,2	9,6	11,2	11,3	17,1	14,2	13,9	15,5
Campylobacter	4,0	5,2	6,1	5,6	3,2	5,9	6,2	4,4	2,9	4,8	5,9	4,3	3,1	5,2	6,9	4,1	3,9
Norovirus	22,6	7,9	6,3	13,0	18,8	14,2	7,5	16,6	23,9	10,9	6,7	12,3	13,6	0,8	0,6	1,0	1,1
Rotavirus	4,9	7,7	1,3	1,6	2,1	4,6	2,5	2,1	3,6	5,7	2,2	1,6	2,3	1,4	2,7	1,2	2,8
No. tested (N)																	
Adenovirus	1039	689	761	779	902	728	849	841	962	698	866	769	686	394	575	511	401
Astrovirus	440	290	372	322	380	354	437	405	484	370	437	424	304	207	291	256	183
C. difficile-Toxin A&B	3699	2770	2975	2707	3030	2588	2958	2598	3104	2607	2889	2731	2656	2116	2617	2214	1768
Campylobacter	8494	6600	8034	7320	8142	7272	8273	7515	8018	6804	8199	7472	6966	5138	6726	5571	4464
Norovirus	4052	2492	2431	2469	2975	2326	2428	2456	3166	2309	3338	2814	2526	1649	2078	1640	1283
Rotavirus	1192	784	946	929	1076	855	985	1020	1182	870	1010	915	812	492	693	571	463

*Data represent the positivity-ratios (plain background) and the number of tests performed per quarter (dotted background) of the respective assay for adenovirus, astrovirus, norovirus, rotavirus, *Campylobacter*, and *C. difficile* toxin A/B. The mean positivity-ratio in pre-pandemic - quarters (PPQ; darker background) were compared to the pandemic quarters (PQ; lighter background) using Mann-Whitney test. Differences of mean positivity-ratios between PPQ and PQ were statistically significant for adeno- (p=.0046), astro- (p=.0032), and norovirus (p=.0032) (striped background) and statistically non-significant for rotavirus (p=.31), *Campylobacter* (p=.91), and *C. difficile* Toxin A&B (p=.17).

Data for tests performed and positive tests were extracted for each quarter from the laboratory information system using Hybase (EpiNet, Bochum, Germany) for the period starting 01/01/2017 through 31/03/2021. Positive data are expressed as positivity ratios, i.e. percentage of positive tests per one hundred tests performed, as this is more robust when dealing with seasonal or other modest changes in number of test requests. Statistical analysis was performed using Abacus 2.0 (Labanalytics, Jena, Germany) using Mann-Whitney test.

Results

The positivity-ratio of norovirus, adenovirus and astrovirus antigen assays were 3–20 fold (and statistically significantly) lower between the periods 1Q/17 through 1Q/20 (pre-pandemic quarters: PPQ) and 2Q/20 through 1Q/21 (pandemic quarters: PQ; Table I, Figure 1). The ratio of genogroup 1 and 2 noroviruses detected by our RT-PCR assay was not statistically significantly different in pre-pandemic quarters as compared to pandemic quarters (data not shown). This makes it unlikely that the drastic drop in norovirus prevalence is due to appearance of a new predominant norovirus genogroup not detected by our RT-PCR. The mean positivity-ratio was lower in PQ compared to PPQ for rotavirus (Table I; p=.31), but failed to reach statistical significance, while for campylobacter antigen (Table I; p=.91) and *C. difficile* Toxin A/B (Table I, Figure 1; p=.17) the mean positivity-ratio was even higher in PQ compared to PPQ. The request numbers in different age groups were analysed, but there were no gross changes in the age distributions between PPQ and PQ (data not shown) and therefore could be excluded as a potential confounding factor.

During the pandemic, the number of test requests was reduced in PQ as compared to the previous PPQ, but generally by less than twofold (Table I). This could have been a strong confounder particularly when resources were overstretched in medical facilities and laboratories alike during the pandemic. For instance, handling SARS-CoV-2 patients and diagnostic requests may lead to testing of only the particularly ill patients for agents of diarrhoea. If this hypothesis would hold true, a significant increase of the positivity-ratios would be expected. However, the opposite was the case for noro-, adeno-, and astrovirus. Additionally, when data were stratified according to inpatient and outpatient, changes in positivity-ratios were seen in similar fashion (data not shown). The relative request numbers were higher for *Campylobacter* antigen in outpatients and higher for norovirus and *C. difficile* toxin A/B in inpatients, respectively, but these differences were reasonably similar in PPQ vs. PQ to exclude bias through changes in overall request habits (data not shown).

Discussion

It appears that introduction of anti-coronavirus lockdown and legal requirements for distancing, wearing of medical facemasks, hygienic measures (like frequent hand washing), closure of shops, restaurants and cultural facilities has led to a significant effect on the incidence of common agents of diarrhoea internationally. Our study demonstrated decreased rates of norovirus, astrovirus, and adenovirus.

In contrast, positivity-ratios of *Campylobacter* infections and toxogenic *C. difficile* diarrhoea were slightly higher in PQ though statistically non-significant and therefore lockdown

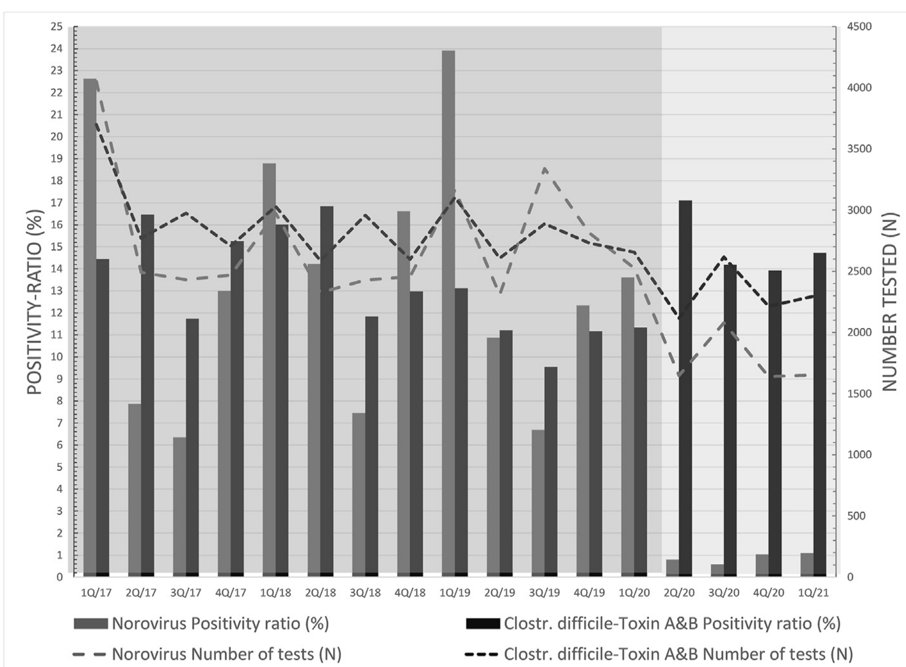

Figure 1. Positivity ratios of assays for norovirus and *C. difficile* Toxin A&B in pre-pandemic and pandemic quarters. Data represent the positivity-ratio (columns) and the number of tests performed per quarter (broken lines) of the respective assay for norovirus and *C. difficile* toxin A/B. The mean positivity-ratios in pre-pandemic - quarters (PPQ; darker background) were compared to the pandemic quarters (PQ; lighter background) using Mann-Whitney test. Differences of mean positivity-ratios between PPQ and PQ were statistically significant for norovirus (p=.0032) and statistically non-significant for *C. difficile* Toxin A&B (p=.17).

measures are apparently not sufficient to detectably decrease transmission of these agents. In Korea, a significant decrease in cases of norovirus (40%), adenovirus (13.4%), rotavirus (32%), sapovirus (12.2%), and astrovirus (7%) were reported, while *Campylobacter*, *Clostridium perfringens*, and *Salmonella* gastroenteritis cases were increased in some periods of 2020 [7]. In Victoria, Australia, the enterovirus specimen positivity-rate was reduced by 84.2% in 2020, while the norovirus outbreak positivity rate declined by 49.0% compared to the previous decade [8].

Other studies concentrated on norovirus incidence alone. Eigner *et al*. [5] reported almost total disappearance of norovirus cases in a similar single laboratory setting as in this study in a different region of Germany. In England, a > 84% decrease in reported norovirus outbreaks was observed [12]. In April 2020 the number of reported norovirus outbreaks declined dramatically in 9 US states. That decline in norovirus outbreaks was significant for all 9 states, and underreporting and/or seasonality are unlikely to be the primary explanation for these findings [6].

All investigated viral agents are transmitted by the faecal – oral route, which is due to excretion of extremely high numbers from 10E9 up to 10E13 particles/g stool of astro-, rota- and norovirus leading to frequent outbreaks in hospital and community settings [9–11]. It seems reasonable to assume that the combination of mandatory wearing of facemasks, frequent hand hygiene and social distancing may interrupt the transmission pathways of these enteric viruses, providing a pronounced effect especially on noro- and astrovirus, which have almost disappeared [this study, 5–8, 12]. When the cycle of epidemic transmission has been broken and these agents are eliminated from a community it is reasonable to speculate that these, and in particular norovirus, may remain on a low level for an additional period after the coronavirus pandemic has been overcome.

However, for *Campylobacter* the main source of transmission is contaminated food [13], which is still consumed despite extended hygienic measures. The lack of a protective effect for *C. difficile* infection is less obvious. Patients with *C. difficile*-associated diarrhoea excrete large numbers of spores which can be transmitted to other individuals via the faecal – oral route and via fomites and contaminated surfaces. Also, asymptomatically colonised individuals excrete spores, which may be infectious [14]. In hospitalised patients, 20–40 % carry *C. difficile* in their intestines after a period of 3 weeks [15]. More recently, it transpires that in *C. difficile* pathogenesis, disturbance of the host microbiome is essential [16] which occurs through antibiotic therapy or extended abdominal surgery and transmission alone may not be enough to trigger disease and therefore initiate diagnostic testing for *C. difficile*-associated diarrhoea [15–17]. Colonisation of patients may still happen despite extended periods of lockdown and hygiene measures, which only in conjunction with additional disturbance of the gut normal flora will lead to overt disease.

Our study has the strength that analysis of effects of anti-coronavirus measures was extended to incidence of non-notifiable diarrheal diseases like astro- and adenovirus and *C. difficile*. The total number of samples examined longitudinally is essential as in crises like the current pandemic diagnostic request-habits may change dramatically and what is not looked for will not be detected. Although this is a single centre study, the number of requests are high enough to allow valid conclusions.

Conclusions

Our study (and those of others) indicate that it seems worthwhile to investigate the effect of anti-coronavirus measures on the prevalence of other communicable diseases besides respiratory diseases [5–8,12]. Of note, hygienic measures used to contain the SARS-CoV-2 pandemic have differential effects on incidence of diarrhoea viruses as compared to bacterial gastrointestinal agents, particularly *C. difficile*, which may lead to revision of measures implemented against this important cause of nosocomial diarrhoea.

Credit author statement

DM, PG, JP, BZ: Conceptualization, Methodology.
DM, JP, BZ: Software.
All authors: Validation.
DM, PG, JP, BZ: Formal analysis, Data Curation, Visualization.
DM, PG, OH, JP, BZ: Resources, Supervision.
All authors: Investigation, Writing - Original Draft, Writing - Review & Editing.

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Conflict of interest statement

DM, PG, MK, DS, UK, OH, JP, and BZ are employees of Bioscientia Labor Ingelheim, Bioscientia MVZ Jena and Bioscientia MVZ Nordrhein, respectively, who performed testing for this study. DM received lecture fees from DiaSorin.

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