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Systematic review with meta-analysis: SARS-CoV-2 stool testing and the potential for faecal-oral transmission

Amarylle S. van Doorn[1,2] | Berrie Meijer[2,3] | Chris M. A. Frampton[4] | Murray L. Barclay[1] | Nanne K. H. de Boer[2]

[1]Departments of Gastroenterology & Clinical Pharmacology, Christchurch Hospital, Canterbury District Health Board and University of Otago, Christchurch, New Zealand

[2]Department of Gastroenterology and Hepatology, AG&M Research Institute, Amsterdam University Medical Centre, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

[3]Department of Gastroenterology and Hepatology, Noordwest Ziekenhuisgroep Alkmaar, the Netherlands

[4]Department of Biostatistics & Medicine, Christchurch Hospital, Canterbury District Health Board and University of Otago, Christchurch, New Zealand

Correspondence
Nanne K. H. de Boer, Department of Gastroenterology and Hepatology, AG&M Research Institute, Amsterdam University Medical Centre; Amsterdam, the Netherlands.
Email: KHN.deBoer@amsterdamumc.nl

Summary

Background: Since the start of the COVID-19 pandemic, there have been many scientific reports regarding gastrointestinal manifestations. Several reports indicate the possibility of viral shedding via faeces and the possibility of faecal-oral transmission.

Aims: To critically assess the clinical relevance of testing stool samples and anal swabs and provide an overview of the potential faecal-oral transmission of SARS-CoV-2.

Methods: A systematic literature search with MeSH terms was performed, scrutinising the Embase database, Google scholar, MEDLINE database through PubMed and The Cochrane Library, including articles from December 2019 until July 7 2020. Data were subsequently analysed with descriptive statistics.

Results: Ninety-five studies were included in the qualitative analysis. 934/2149 (43%) patients tested positive for SARS-CoV-2 in stool samples or anal swabs, with positive test results up to 70 days after symptom onset. A meta-analysis executed with studies of at least 10 patients revealed a pooled positive proportion of 51.8% (95% CI 43.8 - 59.7%). Positive faecal samples of 282/443 patients (64%) remained positive for SARS-CoV-2 for a mean of 12.5 days, up to 33 days maximum, after respiratory samples became negative for SARS-CoV-2. Viable SARS-CoV-2 was found in 6/17 (35%) patients in whom this was specifically investigated.

Conclusions: Viral shedding of SARS-CoV-2 in stool samples occurs in a substantial proportion of patients, making faecal-oral transmission plausible. Furthermore, detection in stool samples or anal swabs can persist long after negative respiratory testing. Therefore, stool sample or anal swab testing should be (re)considered in relation to decisions for isolating or discharging a patient.

As part of AP&T's peer-review process, a technical check of this meta-analysis was performed by Dr Y Yuan. The Handling Editor for this article was Professor Jonathan Rhodes, and it was accepted for publication after full peer-review.

1 | INTRODUCTION

Since December 2019, the world has been dealing with the outbreak of the novel Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) leading to Corona Virus Disease 2019 (COVID-19) that emerged in Wuhan, China. The outbreak in this city led to a major world crisis, the COVID-19 Pandemic.[1,2]

SARS-CoV-2 is a non-segmented positive-sense RNA virus causing the third betacoronavirus outbreak of this century, which appears to have a higher transmission rate but is less deadly than the previous two; SARS-CoV 2003 and Middle East Respiratory Syndrome (MERS) 2012.[3,4] Prior studies demonstrated that the genome sequence of SARS-CoV-2 is 79.5% identical to SARS-CoV, whereas it shares 96.2% of its identity to the Coronavirus RaTG13 found in bats, but the intermediate reservoir has yet to be identified.[5]

While patients infected with SARS-CoV-2 typically present with fever and respiratory symptoms, a rapidly increasing number of studies report patients presenting with a variety of gastrointestinal symptoms such as diarrhoea, vomiting and abdominal pain.[6]

The established transmission route of SARS-CoV-2 is through respiratory droplets (aerosols), mainly during close person-to-person contact,[7] whereas numerous reports also mention the transmission by infected surfaces. Based on the spread through aerosols, the diagnosis of active COVID-19 infection primarily relies on the detection of SARS-CoV-2 viral RNA in specimens from the upper respiratory tract (URT; nasopharyngeal and oropharyngeal cavity) and/or lower respiratory specimens (LRT; sputum and/or bronchoalveolar lavage).[8,9]

Knowledge about SARS-CoV-2's other potential routes of transmission and the significance of different methods of testing is relatively sparse,[10] partly as a result of the novelty of this virus. However, there is a growing body of studies in which SARS-CoV-2 RNA was detected in stool samples (including anal swabs) from COVID-19 patients.[11] These findings support the possibility of a faecal-oral route of transmission. Interestingly, stool tests seem to remain positive when respiratory tests are, or have become, negative.[12-14]

A few articles have briefly reviewed the rapidly increasing body of knowledge on the potential for faecal-oral transmission.[11,15,16]

This study aims to (1) critically assess the clinical relevance of testing stool samples and anal swabs and (2) provide a critical overview of the available literature regarding the faecal-oral transmission of SARS-CoV-2.

2 | METHODS

2.1 | Literature Search

This systematic literature search was performed following the PRISMA guidelines and conducted using the Embase database, Google scholar, The MEDLINE database through PubMed and The Cochrane Library from the outbreak in December 2019 until the 17 June 2020. The search strategy can be found in Online Supplement 1.

All articles were imported to Mendeley (version 1.17.6), and duplicates were removed. Extensive cross-checking of reference lists of the included articles and other reviews was performed. As a result of the rapidly evolving research field concerning COVID −19, we also included journal pre-proof articles.

2.2 | Study selection

All articles were screened based on title and abstract. Studies were included when the following inclusion criteria were met:

1. Study population: Human COVID-19 patients (both adult and paediatric patients) tested for COVID-19 in gastrointestinal specimens (eg stool samples or anal swabs);
2. Study design: case reports/case series, cohort studies, case-control studies and randomised controlled trials.

We excluded articles written before December 2019, when the article or abstract/outcomes were not available in English, Dutch or German and when the results or quality of data were ambiguous. Papers written in Chinese, of which the abstract contained sufficient data to provide answers to our research questions, were included for analysis and data extraction. We excluded articles in which follow-up data were insufficient (ie when results of stool testing were not mentioned). Review articles were not included, however, reference lists were scrutinised for additional articles.

2.3 | Data extraction

We collected the following data from the eligible original articles: study design, geographic location, study period, number of patients, age, types of tested specimens, number of tested specimens, methods of the performed tests, duration and prevalence of positive test results in different specimens, disease severity, gastrointestinal symptoms, endoscopic results, specific evidence supporting faecal-oral transmission and remarkable patient/population characteristics.

Data were subsequently analysed with descriptive statistics. Relevant data were tabulated with a subdivision by study population size. All studies with population of at least 10 patients were included in the meta-analysis.

2.4 | Statistical analysis

A weighted pooled estimate of the proportion testing positive from the stool samples was calculated using the Freeman-Tukey arcsine square root transformation under a random effects model. This analysis was undertaken using MedCalc® v19.4.0. The heterogeneity

in the estimates between studies was statistically tested using Cochran's Q statistic and summarised as I^2.

3 | RESULTS

3.1 | Search Strategy

The search strategy resulted in 300 articles suitable for title and abstract screening. After the exclusion of articles which met the exclusion criteria, we included a total of 95 articles for final analysis. Figure 1 shows details of the selection procedure.

The majority of the included studies were performed in China (74 (77%)), other studies were conducted in Korea (6), Singapore (2), the United States of America (5), Italy (4), France (1), Germany (1), Thailand (1) and Austria (1). All included studies had a case report/case series design. In most study populations, the subpopulation on which stool and/or anal testing were conducted was considerably lower. In total, stool samples or anal swabs (from now on collectively named as GI specimens) from 2175 patients were tested for SARS-CoV-2 RNA. Four studies were included for qualitative analysis, but due to the lack of necessary (follow-up) information, these studies were excluded before final quantitative analysis. [17-20] Therefore, 2149 patients were included for final analysis.

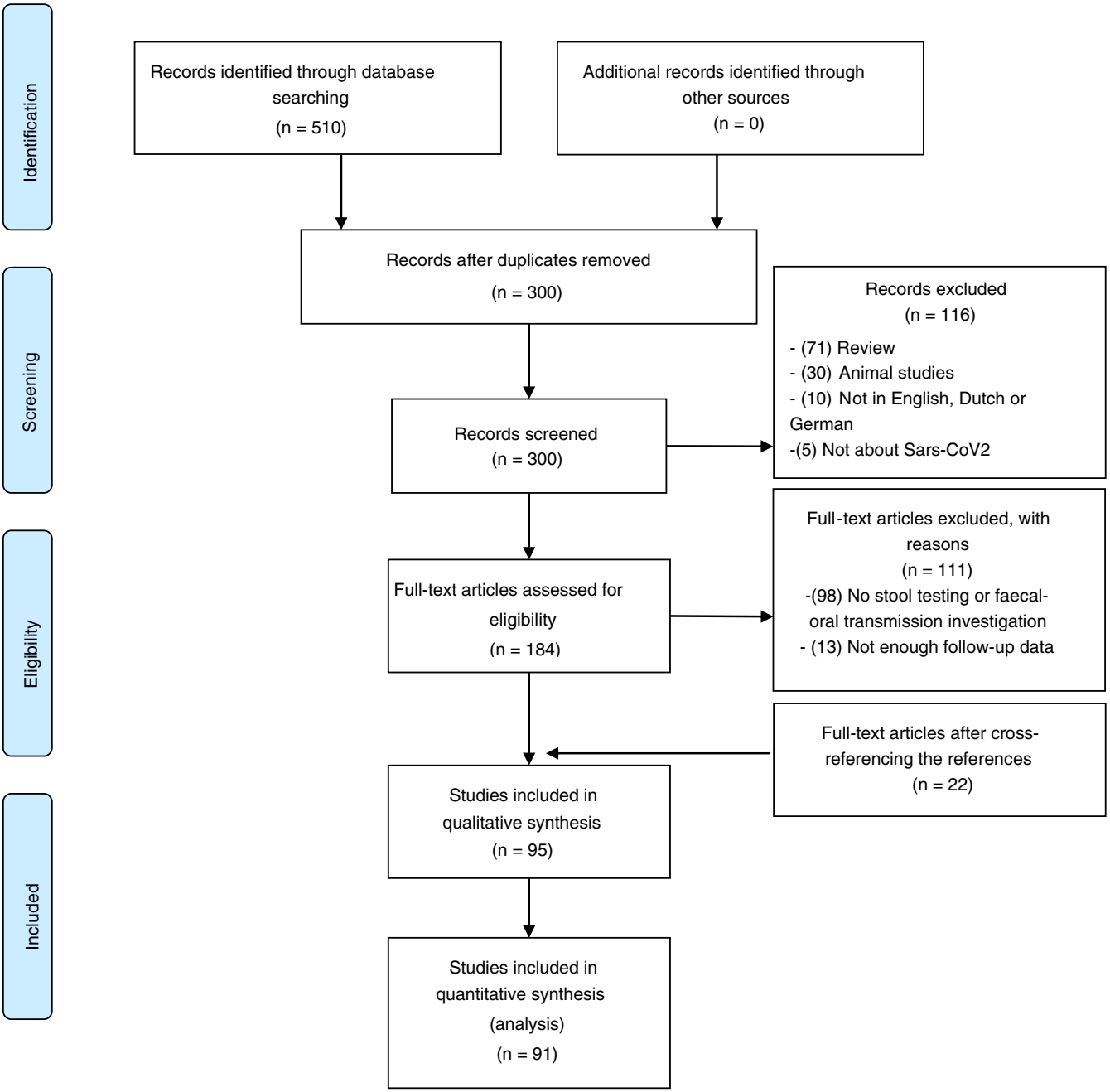

FIGURE 1 Flowchart of included articles

TABLE 1 All studies with study population less than 10 Covid-19 patients

Study	Country of origin	Number of patients included	Age of included patients Average ± (SD) or median (range) in years	Type of GI specimens (S-stool sample A-anal swab)	Number of positive patients (GI specimens) $N_{positive}/N_{total}$ (%) CT mean (SD)	COVID-19 diagnosis based on	Positive stool but (converted) Negative respiratory test $N_{positive\ S\ negative\ O}/N_{total\ positive\ S}$ (%)	Max. Duration positive stool (d)	Time differences between negative respiratory test and negative stool test (d) Mean (range)
Cai[29]	China	6	6.2 (0.25-10.9)	S	5/6 (83)	URT and/or LRT[a] PCR	5/5	30	12 (11-18)
Tang[24]	China	1	10	S	1/1	NS	1/1	25	NS
Young[58]	Singapore	8	47 (31-73)	S	4/8 (50)	NPS PCR	1/4 (25)	NS	4
Chan[59]	China	4	50 (10-66)	S	0	NS	0/4	NA	NA
Kam[30]	Singapore	1	0.5	S	1/1	NPS PCR	NS	9	NA
Zhang Y[21]	China	1	NA	S	1/1	NS	NA	15	NA
Zhang JF[60]	China	1	54	S	1	NPS PCR	NS	25	NS
Zhang B[31]	China	7	26 (0.83 - 35)	A	6/7 (86)	URT and/or LRT[a] PCR	5/6 (83)	44	21.3 (14-31)
Holshue[38]	USA	1	35	S	1/1	NPS PCR	0	NA	NA
Park JY[32]	Korea	1	10	S	1/1	NS	1/1	17	4
Yang Z,[17 b]	China		NA	S	7/7	NS	3/7 (43)	NS	6 (3-7)
Zeng,[61 b]	China	1	Neonate	A	1/1	NS	1/1	NS	NS
Zhang T[62]	China	3	7.7 (6-9)	S	3/3	URT and/or LRT[a] PCR	3/3	10	19 (17-21)
Jiang[25]	China	1	8	A	1/1	NS	2/2	42	NS
Li J[33]	Korea	1	0.67	A	1/1	NS	1/1	14	NS
Wu Y[63]	China	9	26 - 40	Maternal S	1/9	NPS PCR	NA	NA	NA
Lei[64]	China	7	43.2 (14.0)	S	4/7 (57)	URT and/or LRT[a] PCR	2/4 (25)	16	5 - 6
Xing YH[65]	China	3	4.2 (1.5-6)	S	3/3	URT and/or LRT[a] PCR	3/3	30	16 (8-20)
Fan[66]	China	1	0.25	A	1/1	NPS PCR	1/1	28	14
Chen Equation [67]	China	1	34	S	0	Clinical suspicion	NA	NA	NA
Chen L[26]	China	1	25	S	1/1	NS	1/1	11	NS
Lescure[34]	France	5	47 (31-80)	S	2/5 (40)	URT and/or LRT[a] PCR	½ (50)	19	7
Nicastri[68]	Italy	1	Late 20s	S	1/1	URT and/or LRT[a] PCR	0/1	13	NA
Peng[69]	China	9	38.9 (27-62)	A	2/9 (22)	URT and/or LRT[a] PCR	NA	NA	NA
Song[70]	China	1	Middle aged	A	0	NS	NA	NA	NA
Tan LV[71]	China	1	73	A	1/1	NS	1/1	23	7
Xie C[72]	China	9	34 (18-62)	S	8/9 (89)	URT PCR	NA	NA	NA
Thammathiwat[73]	Thailand	1	58	S	1/1 (100)	NPS PCR	NA	NA	NA
Zou B[74]	China	2	2, 13	S	2/2 (100)	Serology	2/2 (100)	24	NS
Shen[75]	China	7	51 (15-88)	S	6/7 (86)	URT PCR	NA	29	NA
Zhou Y[76]	China	9	53 (37-70)	S	9/9 (100)	NPS PCR	NA	NA	NA
Chen X[77]	China	1	7	S	1/1 (100)	NPS PCR	0	5	NA

(Continues)

TABLE 1 (Continued)

Study	Country of origin	Number of patients included	Age of included patients Average ± (SD) or median (range) in years	Type of GI specimens (S-stool sample A-anal swab)	Number of positive patients (GI specimens) $N_{positive}/N_{total}$ (%) CT mean (SD)	COVID-19 diagnosis based on	Positive stool but (converted) Negative respiratory test $N_{positive\ S\ negative\ O}/N_{total\ positive\ S}$ (%)	Max. Duration positive stool (d)	Time differences between negative respiratory test and negative stool test (d) Mean (range)
Kim JY[78]	Korea	2	35, 55	S	0/2	NPS PCR	NA	NA	NA
Liu[79]	China	9	NS	S	8/9 (89)	NPS PCR	8/8 (100)	46	23
Wang Q[80]	China	5	42 (35-56)	S	5/5 (100)	NPS PCR	NA	30	NA
Huang R[81]	China	2	35, 54	A	1/2 (50)	URT PCR	NA	NA	NA
Zhou J[47]	China	1	68	S	1/1	NS	NA	NA	NA
Yin[82]	China	8	54 (40 - 72)	S	8/8	Laboratory confirmed	NA	NA	NA
Mao[83]	China	1	1.2	S	1/1	NPS PCR	NA	28	NA
Wang X[84]	China	3	31.6 (24 - 40)	S	3/3	URT PCR	3/3	40	10.3 (11 - 15)
Xu T[27]	China	1	NA	S	1/1	NS	1/1	NA	NS
Hu[85]	China	3	NA	S	3/3	NPS PCR	2/3 (67)	29	5-15
Tan Y[86]	China	4	8 (3-9)	S	3/4 (75)	URT PCR	1/3 (33)	17	10
Han[87]	Korea	1	27d	S	1/1	NPS PCR	1/1	18	1
Xing Y[88]	China	3	NA	S	3/3	NPS PCR	3/3	NA	16 (8-20)
Cozzi[39]	Italy	2	46-71	S	1/2	NPS PCR	0	NA	NA
Wang C[89]	China	1	50	S	1/1	NPS PCR	1	35	22
Wölfel[48]	Germany	9	NA	S	8/9 (89)	NPS PCR	6/8 (75)	NA	NS

Note: Abbreviations: URT, Upper respiratory tract; LRT, Lower respiratory tract; SP, Sputum; OS, Oral Sample; NPS, Nasopharyngeal Sample; TS, Throat Swab; NA, Not Applicable; GI, Gastrointestinal; PCR, polymerase chain reaction; SD, standard deviation; NS, not specified

[a]World Health Organization Guidance recommends collection of upper respiratory tract (URT) specimens (nasopharyngeal and oropharyngeal) and, where clinical suspicion remains and URT specimens are negative, to collect specimens from the lower respiratory tract (LRT) when readily available (expectorated sputum, or endotracheal aspirate/bronchoalveolar lavage in ventilated patient).

[b]Data extracted from abstract; full-article only available in Chinese.

In 23 studies, only children were included, of which four studies did not specify the age of included children. In 43 studies, only adults were included, whereas 12 studies included both children and adults. In the remaining 17 studies, the range of age was not reported. Detailed study characteristics are depicted in Tables 1 and 2, with subdivision by study population size (Table 1: n < 10 and Table 2: n ≥ 10).

3.2 | Test Characteristics

Seventeen (18%) studies tested SARS-CoV-2 presence in anal swabs and 81 (85%) in stool samples. In three studies, both specimens were tested. In all studies but one, real-time reverse transcription polymerase chain reaction (RT-PCR) was used to detect SARS-CoV-2. One study performed inoculation of stool suspension into Vero cells followed by virus detection through electron microscopy.[21]

3.3 | Outcomes

In 91/95 (96%) of the included studies, SARS-CoV-2 RNA was identified in GI specimens from at least one of the included patients (Tables 1 & 2). In total, 934 patients had one or more positive GI specimens (43%). A meta-analysis performed on studies with at least 10 patients showed a pooled positive proportion of 51.8% (95%CI 43.8 - 59.7%; Figure 2; Supplementary Table 1). It has to be mentioned that there is a significant amount of heterogeneity among the included studies, with an I^2 of 91.9%. SARS-CoV-2 RNA was detected in GI specimens up to a maximum of 70 days after the onset of symptoms and 26 days after discharge from hospital.[22,23] In total, 42 studies reported the maximum days of GI specimen positivity after symptom onset or first positive test in any specimens, with a mean of 25.0 (range 3-70) days after symptom onset.

In 22 patients (1%), infection with COVID-19 would not have been diagnosed without GI specimens testing, meaning these

TABLE 2 All studies with study population more than 9 Covid-19 patients

Study	Country of origin	Number of patients included	Age of included patients Average ± SD/ median (range) in years	Type of GI specimens (S-stool sample A-anal swab)	Number of positive patients (GI specimens) $N_{positive}/N_{total}$ (%) CT mean (SD)	COVID-19 diagnosis based on	Positive stool but (converted) Negative respiratory test Npositive S negative O/ Ntotal positive S (%)	Max. Duration positive stool (d)	Time differences between negative respiratory test and negative stool test (d) Mean (range)
Wang W[19]	China	153	44 (5-67)	S	44/153 (29)	"Based on symptoms and radiology and confirmed by SARS-CoV-2 detection"	Yes[α]	NA	NS
Zhang JC[40]	China	14	41 (18-87)	S	5/14 (36)	NS	3/5 (60)	13	NS
Zhang W[35]	China	16	NA	A	10/16 (63)	NPS PCR	6/10 (60)	NA	NS
Xiao F, Tang M[41]	China	73	43 (0.83-78)	S	39/73 (53)	NPS PCR	17/39 (44)	12	NS
Kujawski[42]	USA	10	53 (21-68)	S	7/10 (70)	NPS, URT and/ or LRT[a] PCR	NS+ 3/7 (43) mean 3.3 (0-5) days difference ORS+ 5/7 (71) mean 3.2 (0-13)	25	NS
Ling[12]	China	66	44.0 (34.0-62.0)	S	55/66 (82)	NS	43/55 (78)	16	2.0 (1.0-4.0)
Chen W[90]	China	28	NA	A	11/28 (39)	NS	Yes[α]	Max. 13	NS
Wu Yongjian[13]	China	74	41.29 ± 3.14	S	41/74 (55)	URT and/or LRT[a] PCR	32/41 (78)	47	Mean: 11.2 (1- 33)
Xu Y[43]	China	10	6.6 (0.17-15)	A	8/10 (80)	NPS PCR	8/8	26	17 (2-19)
Han[44]	China	22	43.3 (27-71)	S	12/22 (55)	NS	NA	NA	NA
Chen Chen[36]	China	19	36.5 (2-64)	S	12/19 (63)	NPS PCR	9/12 (75)	24	4.7 (1-10)
Lin Lu[45]	China	65	45.3 ± 18.3	S	31/65 (48)	NPS PCR	NA	NA	NA
Chen Y[14]	China	42	51 (42.75-62)	S	28/42 (67)	URT and/or LRT[a] PCR	18/28 (64)	23	7 (6-10)
Cheung[46]	China	59	58.5 (22-96)	S	9/59 (15)	NS	NA	NA	NA
Tan X,[18 b]	China	13	Children	S	NA	NS	Yes[α]	NA	12
Wu J[20]	China	NS	66.7 ± 9.1 years	A & S	132 patients Total of tests: A+ 12/120 (10) S+ 24/244 (10)	NPS PCR	Yes[α]	NS	NS
Ma X[37]	China	27	6 children 4.7 (0.92-9) 2 adults 33 and 39	S	8/27	NS	8/8	35	14.6-27.4
Pan Y[91]	China	17	NA	S	9/17 (53)	NS	NA	NA	NA
Lo[92]	China	10	54 (27 - 64)	S	10/10	URT and/or LRT[a] PCR	2/10 (20)	19	2 - 3
Xiao Fei, Sun J[49]	China	28	NA	S	12/28 (43)	NS	NA	NA	NA

(Continues)

TABLE 2 (Continued)

Study	Country of origin	Number of patients included	Age of included patients Average ± SD/ median (range) in years	Type of GI specimens (S-stool sample A-anal swab)	Number of positive patients (GI specimens) $N_{positive}/N_{total}$ (%) CT mean (SD)	COVID-19 diagnosis based on	Positive stool but (converted) Negative respiratory test Npositive S negative O/ Ntotal positive S (%)	Max. Duration positive stool (d)	Time differences between negative respiratory test and negative stool test (d) Mean (range)
Yuan[28]	China	78	Single A+ 6.2 (2.7-8.3) Single TS+ 7.5 (3.3-11.7)	A	41/78 (53)	NPS PCR	17/41 (41)	23	NS
Zhang N[22]	China	12	48.0 (40-62)	S	10/12 (83)	NS	NS	25	26
Zuo[55]	China	15	55 (44-67.5)	S	11/15 (73)	NS	NA	37	NA
Park S[93]	Korea	36	26 (18-57)	S	2/46 (4)	URT and/or LRT[a] PCR	No	50	NA
Deng L[94]	China	56	>18	S	25/56 (45)	NPS PCR	4/25 (16)	7	NS
Wu B,[95 b]	China	36	49 (17-86)	S/A	20/36 (56)	NS	NA	NA	NA
Guan[96]	China	62	68 (44-77)	S	4/62 (6)	URT and/or LRT[a] PCR	1/4 (25)	NA	NS
Szymczak[97]	USA	77	NA	S	27/77 (35)	URT and/or LRT[a] PCR	NA	33	NA
Shi D[98]	China	99	54 (IQR 39-64)	S	21/99 (21)	URT and/or LRT[a] PCR	NA	NA	NA
Mesoraca[99]	Italy	15	NA	S	11/15 (73)	URT and/or LRT[a] PCR	10/11 (89)	40	NS
Chen Z[100]	China	32	9.5 (3mo – 18y)	S/A	17/32 (53)	NPS PCR	NA	65	13.1
Guo[101]	China	23	20-62	S	11/23 (48)	NPS PCR	NA	NA	NA
Deng W[102]	China	61	55	S	17/61 (28)	NPS PCR	14/17 (82)	NA	14
Du[103]	China	10	5 (1-14)	S	7/10 (70)	URT and/or LRT[a] PCR	7/7 (100)	Median 34	25
Hua[23]	China	35	8 (0.25 - 14)	S	32/35 (91)	URT and/or LRT[a] PCR	NA	70	NA
Han[104]	Korea	12	6.5 (0.01-16)	S	11/12 (92)	NPS PCR	NA	NA	NA
De Ioris[105]	Italy	22	7 (0 - 18)	S	15/22 (68)	NPS PCR	6/9 (67)	14	NS
Zhao[106]	China	401	NA	A	80/401 (20)	Clinical suspicion	NA	49	NA
Perchetti[107]	USA	20	NA	S	13/20 (65)	NPS PCR	NA	NA	NA
Lu[108]	USA	28	NA	S	7/28 (25)	NS	NA	NA	NA
Wu Q[109]	China	10	6 (0.10 - 15.1)	S	10/10	NPS PCR	8/10	23	11 (5 - 23)
Zheng[110]	China	96	55 (IQR 44.3-64.8)	S	57/96 (59)	URT and/or LRT[a] PCR	NA	59	NA
Yun[111]	China	32	50 (IQR 37-66)	S	8/32 (25)	NPS PCR	NA	NA	NA
Effenberger[112]	Austria	40	NA	S	12/40 (30)	NPS PCR	NA	NA	NA
Li Y[113]	China	13	52.8 ± 20.2	S	5/13 (83)	NPS PCR	2/5 (40)	24	14-15
Huang J[114]	China	33	47 (2 - 84)	S	30/33 (91)	NPS PCR	NA	NA	NA
Yongchen[115]	China	15	37 (10 - 37)	S	5/15 (33)	NPS PCR	4/5 (80)	30	8 (2-17)

Note: Abbreviations: URT, Upper respiratory tract; LRT, Lower respiratory tract; SP, Sputum; OS, Oral Sample; NPS, Nasopharyngeal Sample; TS, Throat Swab; NA, Not Applicable; GI, Gastrointestinal; PCR, polymerase chain reaction; SD, standard deviation; NS, not specified

[a]World Health Organization Guidance recommends collection of upper respiratory tract (URT) specimens (nasopharyngeal and oropharyngeal) and, where clinical suspicion remains and URT specimens are negative, to collect specimens from the lower respiratory tract (LRT) when readily available (expectorated sputum, or endotracheal aspirate/bronchoalveolar lavage in ventilated patient).

[b]Data extracted from abstract; full-article only available in Chinese.

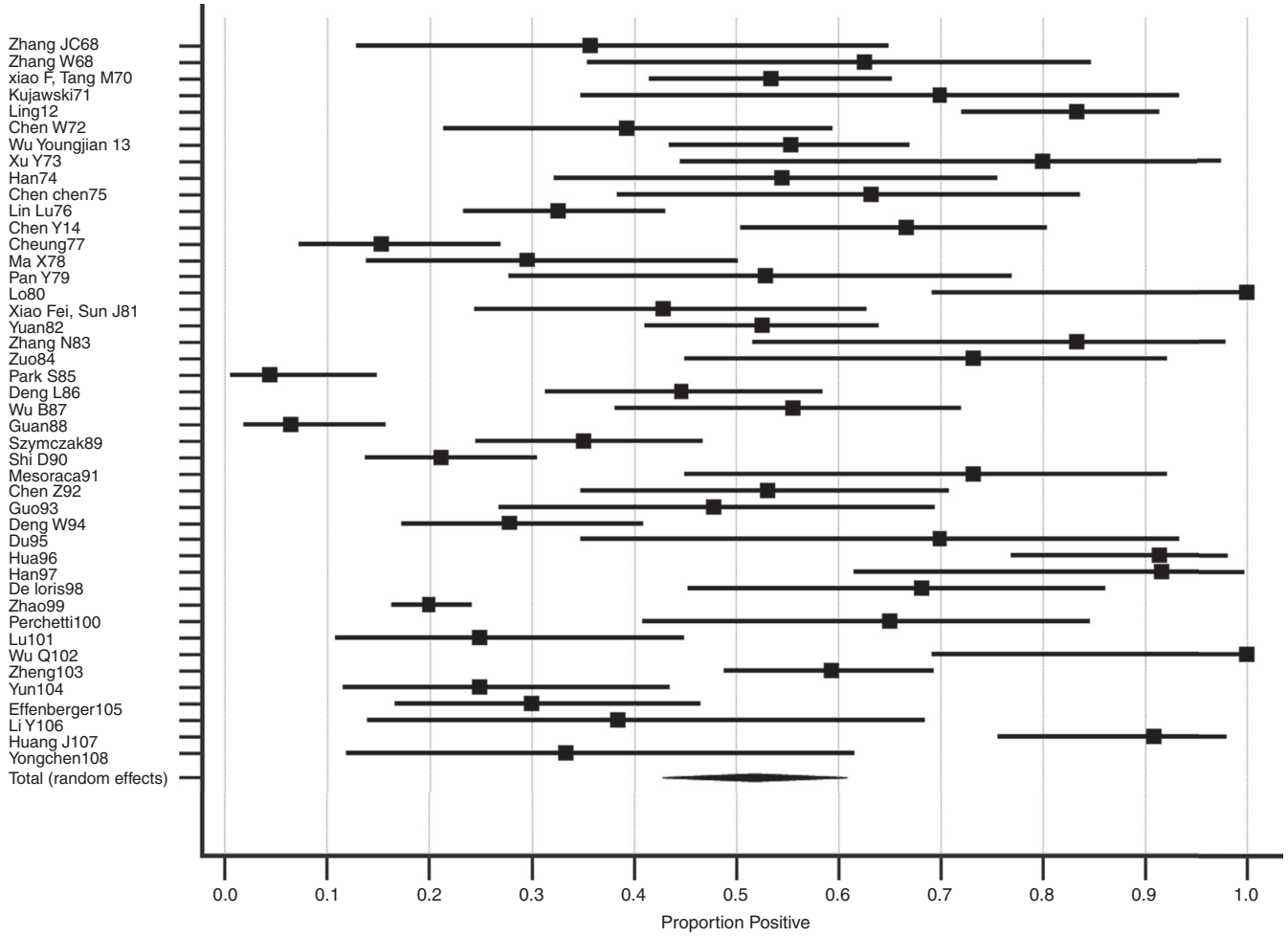

FIGURE 2 Meta-analysis of included articles. The proportion positive shows the number of tests positive for SARS-CoV-2 divided by the total number of tests. Bars show 95% CI indeed. Data are further specified in Supplementary Table 1

patients had negative results in every other specimen type tested and would not have been confirmed as carriers of the virus otherwise.[19,24-28]

Out of 54 studies with serial SARS-CoV-2 RNA test results for both respiratory and GI specimens, 49 (91%) studies reported persistently positive tests for SARS-CoV-2 RNA in GI specimens after respiratory specimens had become negative. Almost two thirds of the patients (282/443 (64%)) who had a positive GI specimen test had persistent positive GI specimen tests despite negative respiratory tests. The mean duration of positive GI testing after negative respiratory testing was 12.5 days. The maximum duration of positive GI testing after negative respiratory testing was 33 days.[13] Interestingly, several studies reported patients with ongoing positive GI specimen tests after hospital discharge.

Detectability of SARS-CoV-2 RNA depends on the type of specimen tested during different stages of the disease (eg respiratory or faecal sample). In most studies in which serial measurements took place, it was reported that viral RNA was more likely to be detected in respiratory tract samples during an early stage of the disease, whereas GI specimens were more likely to be positive later on during the disease.[12-14,22,29-37]

Twelve studies discussed the association between positive GI specimens and GI symptoms.[13,14,19,38-46] In all studies, the majority of patients with GI symptoms tested positive in GI specimens, but the association was not statistically significant in most studies. In the study by Han *et al*, it was observed that patients with GI symptoms were significantly more likely to test positive for SARS-CoV-2 in a stool test ($P = 0.033$).[44] Furthermore, Cheung *et al* found that the proportion of positive stool tests and the stool viral load was higher in patients with diarrhoea than without ($P = 0.019$ and 0.06 respectively).[46]

In addition to the clinical symptoms and the positive GI specimens testing, two studies detected SARS-CoV-2 RNA in endoscopic specimens of the oesophagus, stomach, duodenum and rectum in 1/1 and 2/6 patients.[41,45] Viability of SARS-CoV-2 was investigated and detected in five studies, in which six patients (6/17 (35%)) had live active virus in their GI specimens using Vero cell testing.[19,21,47-49]

4 | DISCUSSION

In this study, we performed a systematic review of the rapidly expanding body of literature to assess the performance and accuracy

of testing stool samples or anal swabs and investigate the potential faecal-oral transmission of SARS-CoV-2. We conclude that the gastrointestinal tract is a potential shedding route of SARS-CoV-2 as all but four of the 95 studies with GI specimens testing found positive results of SARS-CoV-2 RNA by RT-PCR in at least one of the patients. A pooled proportion of 51.4% of all included patients tested positive in GI specimens.

Viral RNA can be detected in GI specimens up to 70 days after onset of symptoms or after the first positive SARS-CoV-2 test in any specimen. After respiratory tests turned negative, GI samples stayed persistently positive up to a maximum of 33 days, implying that the virus may be actively replicating in the patient's gastrointestinal tract and that faecal–oral transmission might occur after viral clearance in the respiratory tract. Although we observed a relation of patients with gastrointestinal symptoms to be more likely to test positive for SARS-CoV-2, the absence of gastrointestinal symptoms is not a firm indicator for negative GI specimen tests.

While SARS-CoV-2 may be shedding through stool in a notable subset of patients, the detection of viral genetic material in stool does not necessarily imply that viable infectious virions are present in GI specimens or that the virus can or has spread through faecal transmission. Live SARS-CoV-2 was found in 6/17 (35%) of the patients in which this was specifically investigated. Isolation of live SARS-CoV-2 in cultured GI specimens underlines the possibility of faecal-oral transmission through infected faeces.

Similar patterns of faecal-oral transmission and the relevance of stool testing of other coronaviridae have been witnessed over the years.[50,51] The initial SARS-CoV outbreak in the Amoy Gardens was primarily attributed to an airborne spread via inefficient sanitation and toilet ventilation systems.[52,53] Infection of the GI tract with the previous coronaviridae is proposed to be mediated via Angiotensin Converting Enzyme (ACE)-2 receptors. ACE-2 has also been identified as the host receptor that interacts with the viral spike protein to facilitate entry of SARS-CoV-2 into the host cell.[50] ACE-2 receptors are highly expressed in the small intestine and the binding affinity of ACE-2 receptors determine infectivity. As ACE-2 modulates intestinal inflammation, SARS-CoV-2 may disrupt ACE-2 function and result in GI shedding and symptoms, such as diarrhoea, vomiting and abdominal pain.

Furthermore, wastewater surveillance and wastewater-based epidemiology are considered a complementary approach to estimate the presence and even the prevalence of COVID-19 in communities, detecting SARS-CoV-2 in wastewater from households with infection.[54] Additionally, a recent study observed prolonged gut microbiome dysbiosis in COVID-19 patients and its association with faecal SARS-CoV-2 virus shedding and disease severity, suggesting that SARS-CoV-2 infection may be associated with a more long-lasting effect on the gut microbiome.[55]

Besides the fact that the genome of both SARS-CoV viridea and thus the shedding routes are very similar, SARS-CoV-2 also falls under the same shell disorder category as SARS-CoV, and SARS-CoV-2 has the hardest outer shell within the entire corona family. The hardness of the outer shell could provide SARS-CoV-2 with greater resilience to conditions outside the body and in bodily fluid, as the harder shell will provide better protection. Chances of infection via indirect contact and airborne virus from faeces and bodily fluids are therefore higher and faecal-oral transmission more likely.[56]

The results of this study may have various consequences for the diagnosis, prognosis and spread of COVID-19. First and foremost, worldwide the decision to isolate or discharge a patient is primarily based on relevant clinical symptoms, focusing on the respiratory tract, and (sequential) negative test results on respiratory specimens collected more than 24 hours apart.[57] We observed that in 64% of patients who tested positive for SARS-CoV-2 in GI specimens, their GI specimens remained positive for a mean of 12.5 days after respiratory samples became negative. As a result, a number of patients were discharged up to a month before the absence of SARS-CoV-2 in GI specimens could be guaranteed. The (additional) use of GI specimen testing may provide a more appropriate rationale for isolation and discharge.

A major concern could be continuing person-to-person transmission by the faecal-oral route, which argues for closer attention to hand and sanitation hygiene. This should be considered when determining diagnosis and isolation policies.

In general the risk to health care professionals from patient exposure is well known, specifically in high aerosol-generating procedures. Currently, medical management protocols include measures to mitigate the aerosol transmission risks from procedures related to respiratory tract.[8] Our analysis suggests that faecal-oral transmission risk from gastrointestinal procedures such as colonoscopies or physical examination, should also be taken into account.

Determining whether a virus is viable using RNA detection by RT-PCR is challenging. Limited studies have observed viable virus in stool and further research is needed to determine whether the irrefutable faecal shedding and the high and long-lasting detection rate of viral RNA in GI specimens really indicates the likelihood of faecal-oral transmission. Studies using fresh stool samples at later time points in patients with extended duration of GI specimen positivity are required to define transmission potential. Nevertheless, the importance of GI specimen tests for detection of SARS-CoV-2 in general, and even more in the longer term surveillance of infected patients, has been confirmed in our systematic review.

All included studies were observational case studies without control groups, based on a relatively small number of heterogeneous patients (I^2 approximately 90%), and the timing of specimen collection has been largely inconsistent and unstandardised. In particular, evidence for viable virions in GI specimens is based on a small number of patients whose specimens were collected at different times over the course of illness or convalescence. This is not surprising, as most included studies are case reports or small case series of patients treated on the frontlines during the pandemic, in which adhering to standard research protocols is difficult. This generates the risk of bias in these kinds of studies, especially publication bias.

As a result, our analyses were based on relatively small patient groups (median 9; range 1-401 patients) and inconsistent methods, parameters, sample timing, sample frequencies and study endpoints differing widely between the included studies, impeding

comparisons and robust conclusions. In the early response to the emerging COVID-19 outbreak, only respiratory specimens were required for the detection of SARS-CoV-2 according to initial clinical guidelines. A lot of studies, therefore, refrained from obtaining GI specimens from the patients during their first few days of hospitalisation or observation and could not determine whether respiratory and GI specimens were positive on RT-PCR analysis simultaneously. Furthermore, the phenomenon that viral RNA of SARS-CoV-2 can remain positive in GI specimens after respiratory samples became negative was not identified in all studies. This resulted in inadequate (follow-up) information, potentially causing a (outcome) measurement bias.

The sole four studies that reported no positive tests in GI specimens were all based on small sample size (1-4 patients) and the testing was performed at an early stage of the disease course. Our review demonstrated that there seems a tendency for SARS-CoV-2 to be more detectable in the respiratory tract at an early stage of the disease and later on, more likely to be detected in GI specimens, which could explain the early negative testing.

Our review confirms that SARS-Cov-2 is commonly present in stool samples or anal swabs in which the virus can persist long after respiratory testing has become negative and that the virus may be viable. This suggests the possibility of faecal-oral transmission and that stool sample or anal swab testing should be (re)considered in relation to decisions for isolating or discharging a patient.

AUTHORSHIP

Guarantor of the article: NdB.

Author contributions: AvD drafted the first version of the manuscript and performed the analyses. BM, MB and NdB critically revised the manuscript for important intellectual content. CF performed the statistical analyses. All authors approved to the final version of the manuscript.

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ORCID

Amarylle S. van Doorn https://orcid.org/0000-0003-2961-2097
Berrie Meijer https://orcid.org/0000-0002-4760-3269
Murray L. Barclay https://orcid.org/0000-0003-2944-8338

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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