Epidemiological approximation of the enteric manifestation and possible fecal–oral transmission in COVID-19: a preliminary systematic review

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The recent appearance of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection has led to the publication of the first evidence on gastrointestinal symptoms (GIS), the possible enteric involvement of the virus and the detection of RNA in stool, with its possible implication in the fecal–oral transmission of coronavirus disease 2019 (COVID-19). We aimed to conduct a systematic review to describe the epidemiological scientific evidence on GIS, enteric involvement and fecal excretion of SARS-CoV-2 viral RNA and to discuss the possible fecal–oral transmission pathway of COVID-19. Eur J Gastroenterol Hepatol XXX: 00–00

Introduction

In early December 2019, a set of cases of pneumonia of an unknown cause was identified in Wuhan (China) [1,2]. China notified the WHO office on 31 December 2019. On 7 January 2020, the Chinese Health Authorities confirmed the identification of a novel betacoronavirus [severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)] from the same family that caused SARS or Middle East respiratory syndrome (MERS). On 30 January 2020, the Director-General of the WHO declared a public health emergency of international concern. On 11 March 2020, the WHO made an address that declared the outbreak caused by the novel betacoronavirus 2 (2019-nCoV) a pandemic [3,4].

There is evidence of the similarity of SARS-CoV-2 with the genetic sequences of different coronaviruses (CoVs) present in at least five species of bats, according to surveillance studies conducted [5,6]. At least three of these species were found in Wuhan, Hubei province, in the center of the People's Republic of China [7], but the bat CoV with which SARS-CoV-2 has the greatest genomic similarity was isolated from *Rhinolophus sinicus* (genetic sequence MG772933), described in ref. [8], which could indicate that this species could also have been the original source of SARS-CoV-2 and probably reached humans after passing from an intermediate host, the civet Paguma larvata or the pangolin Manis pentadactyla [9,10]. Although said genetic similarity has been studied, the epidemiological link must be proven [11]. The way in which the virus could be transmitted from the animal source to the first human cases is unknown.

As of 22 April 2020, when this article was written, the virus had spread, according to cases reported, to 215 countries, territories or reporting areas around the world, as reflected in the WHO SARS-CoV-2 disease (COVID-19) Situation Report-98 published on 27 April 2020. More than 2 878 196 cases and at least 198 668 deaths have been confirmed [12].

It is important to note that the basic reproduction number (R_0), the indicator of transmissibility of SARS-CoV-2, has been estimated at 2.30 from reservoir to person and that person-to-person transmission was 3.58 [13]. Two reviews recorded a total of 32 studies of different methodologies estimating R_0 values ranging from 1.5 to 6.5 during the epidemic in Wuhan [14].

In the absence of specific clinical manifestations, the identification of transmission chains and follow-up of subsequent contacts would be much more complicated, especially if many infected individuals remain asymptomatic, presymptomatic or mildly symptomatic carriers [15].

The clinical manifestations such as dry cough, fever and dyspnea are well known and described. In the first series in Wuhan, 2–10% of patients with COVID-19 had GIS such as diarrhea, abdominal pain and vomiting [16,17]. Gastrointestinal infection is possible, and the mechanism for SARS-CoV infection in the gastrointestinal tract is already known to be the cellular receptor of angiotensin-converting enzyme 2 (ACE2) [18].

To date, several studies have been published that refer to the viral excretion of SARS-CoV-2 in stool and investigate whether fecal SARS-CoV-2 RNA levels correlate with disease severity and the presence or absence of GIS, and, on the other hand, analyzing whether SARS-CoV-2 RNA in stool can also be detected in the incubation or convalescent phases of COVID-19 [19], which could imply possible fecal–oral transmission.

The identification of the main routes of transmission of SARS-CoV-2 infection should be a priority in health research, as it may make it possible to define preventive

European Journal of Gastroenterology & Hepatology 2020, XXX:00–00

Keywords: coronavirus, coronavirus disease 2019, enteric virus, epidemiology, fecal–oral, gastrointestinal, severe acute respiratory syndrome coronavirus-2, systematic review

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Received 15 June 2020 **Accepted** 17 August 2020

strategies to further reduce its burden of morbidity and mortality. Since different occupations, migratory and mobility activities of communities and populations may represent different pathways for acquiring infection. Our aim was through a systematic review of the literature to know the frequency of GIS and describe the fecal excretion of SARS-CoV-2 in patients with COVID19, which could imply possible fecal–oral transmission.

Methods

Search strategy and inclusion criteria

We conducted a systematic search of electronic medical databases (PubMed, Embase and Google Scholar) from 31 January until 12 April 2020 (date of last search) to retrieve published scientific articles assessing or making references to the GIS, gastrointestinal infection, detection of viral RNA in stool and possible enteric or fecal–oral transmission of the SARS-CoV-2 during the COVID-19 global pandemic. The exact query terms used in the database research were: 'coronavirus' OR 'coronavirus' AND 'feces' OR 'feces' OR 'stool' AND 'SARS-CoV-2 transmission' OR 'transmission' AND 'COVID-19' OR 'severe acute respiratory syndrome coronavirus 2' OR 'severe acute respiratory syndrome coronavirus 2' OR '2019-nCoV' OR 'SARS-CoV-2' OR '2019nCoV' OR 'Wuhan' AND 'coronavirus' OR 'coronavirus' AND 'fecal-oral covid-19' OR 'gastrointestinal evidence AND covid-19 transmission' OR 'intestinal participation in covid-19 transmission' OR 'sars-cov-2 AND population transmission' OR 'SARS-CoV-2 AND viral RNA concentrations in stool biological samples' OR 'stool test AND for determination of SARS-CoV-2'. Each reference retrieved was independently examined, following predefined criteria for determining eligibility for the systematic review (Fig. 1).

Inclusion and exclusion criteria

Eligibility criteria included original and editorial articles, comments, letters to the editor, guidelines and case reports in which original results were presented. Research not involving humans (for example, in-vitro or animal research; experimental studies with an evaluation of SARS-CoV-2 infection in gastrointestinal biological samples recovered from laboratory databases) was included. Eligible study designs included randomized, cohort, case-control, cross-sectional, ecological studies and modeling studies.

Exclusion criteria were: documents written in a language other than English, Portuguese, Spanish, French, Italian; previous systematic reviews were not eligible; studies that do not assess or provide the prevalence of GIS in confirmed cases of COVID-19 or studies than not provide information about the isolation or proteins detection of SARS-CoV-2 from stool samples.

Study selection and data extraction

Decisions were made independently by two reviewers using the search strategy for eligible studies, which were

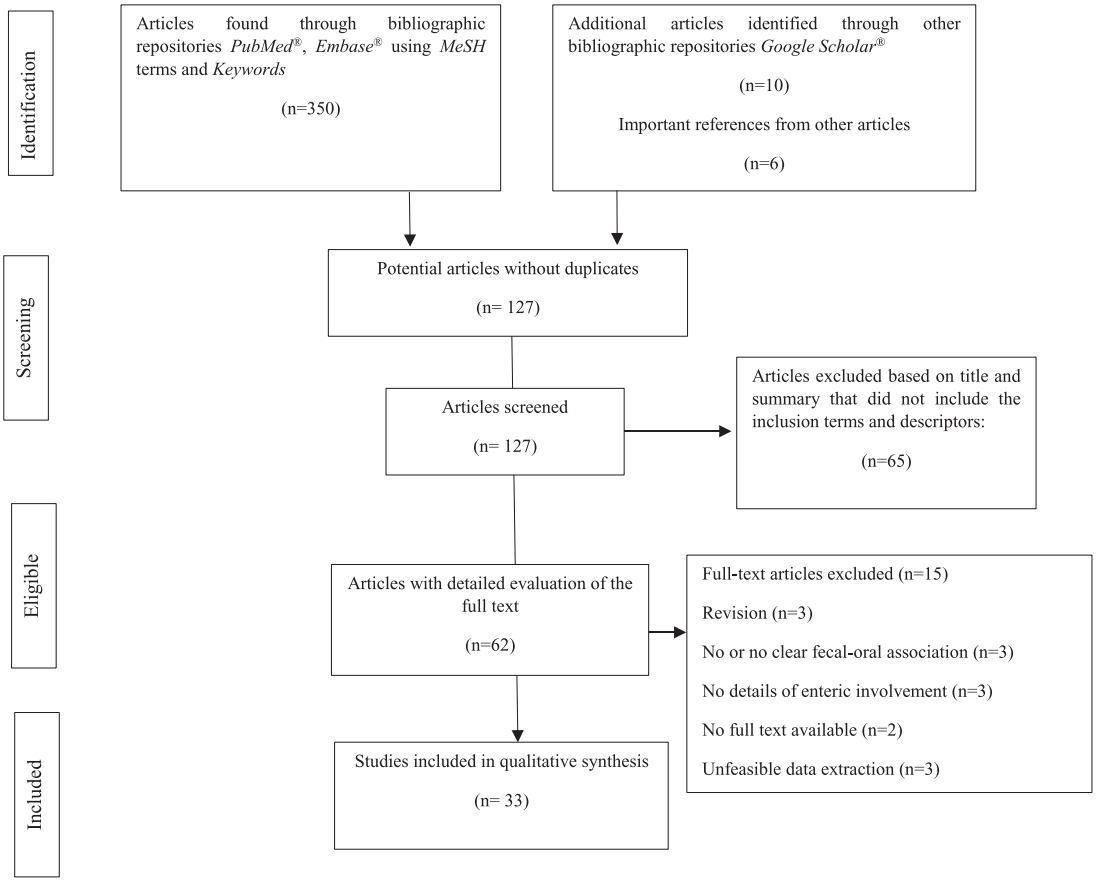

Fig. 1. Four-level flowchart of studies on epidemiological scientific evidence on the possible fecal–oral transmission pathway of SARS-CoV-2 infection (COVID-19). COVID-19, coronavirus disease 2019; SARS-CoV, severe acute respiratory syndrome coronavirus-2.

compared, and discrepancies were resolved by consensus or consultation after discussion with another independent investigator. The retrieved study references were stored in an electronic bibliographic data repository to identify additional relevant publications that were missed in the initial search strategy.

For the extraction of data from the selected articles, a predesigned data collection form was prepared to extract relevant information from the full texts, including the study design, year of publication and the period of data collection.

Quality assessment

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement was used as an instrument for the critical reading and evaluation of cross-sectional epidemiological studies and case series, and those with a focus on prevalence (cohort, case-control and cross-sectional), according to a 22-point checklist related to the different sections of an article: title, summary, introduction, methodology, results and discussion. Of these, 18 points are common to the three study designs: cohort, case-control and cross-sectional; the other four are specific to cohort, case-control or cross-sectional studies [20,21]. The quality of the study was considered HIGH (H) if most summary statements were answered as 'excellent' or 'good'; MEDIUM (M) if internal validity was rated as 'MEDIUM', or most summary statements were rated as 'good' or 'fair'; and LOW (L) if internal validity was rated as 'LOW', or most summary statements were rated as 'fair' or 'poor' [22].

Statistical methods

Considering the heterogeneity in the studies identified through the systematic review and the options for presenting the results in each article, quantitative synthesis of the main findings could not be made. We performed a simple pooled analysis to estimate the frequency of GIS as if all COVID-19 patients were from the same study. The data were combined without being weighted.

Results

Study characteristics

In total, we identified 350 references addressing potentially relevant descriptors of the review (Fig. 1). Of these, 33 (9,4%) articles met our eligibility criteria and were therefore included in the analysis (Table 1). All articles selected according to the review objective were published in 2020 due to the recent emergence of the COVID-19 pandemic, and data collection or surveillance periods of these studies took place between December 2019 and 24 March 2020, mainly in the following regions: Asia (88.8%), Europe (12.1%) and the Americas (9.1%). Studies spanned a wide age range, from pediatric subjects 1 day to 92 years of age, in those studies in which information was provided, including experimental studies. Most studies analyzed were observational 24/33 (72.7%), 8/33 (24.2%) cohort and 1/33 (3%) case-control studies. According to the external quality assessment of the studies, 17/33 (51.5%) had medium quality and 14/33 (42.4%) were of high quality, according to STROBE (Table 1).

General characteristics of the included studies

Due to the novelty in the occurrence of the COVID-19 pandemic, no broad geographical distribution was observed in the studies reviewed, and we included 2/33 (6%) types of articles sent to journals as correspondence articles, letter to the editor or brief review, as they provided data from an original study. Table 1 shows the list of studies according to the inclusion criteria and descriptors used.

The PubMed bibliographic repository was the most widely used for retrieval through open access to available articles. The articles identified were mainly designed as cross-sectional descriptive studies and some observational studies (cohort, cases and controls) with a retrospective or longitudinal design. One case-control study was found, and no ecological or modeling studies or RCT studies were found (Table 1).

As regards sex and age, as another of the sociodemographic variables collected in the different studies included, we found the distribution by sex to be relatively homogeneous in the different studies, with global average distribution of 53.1% male and 46.9% female and varied in a range from pediatric patients of 1 day to adult patients of 92 years of age.

Prevalence of gastrointestinal symptoms in COVID-19

Table 2 shows the presence of GIS, which generally showed great variability between 2 and 100% of all articles reviewed. It also depended on the sample size, which ranged from 1 to 1141 patients included in the different studies. In these studies, the GIS were related to the presence of diarrhea between 1 and 80%, nausea and vomiting between 3.6 and 50% and abdominal pain between 1.9 and 33.3%. According to our pooled analysis, 16.1% presented GIS, 8.3% diarrhea, 12% nausea-vomiting and 4% abdominal pain.

SARS-CoV-2 detection in stool samples

The samples used for diagnosis of SARS-CoV-2 infection, in some studies [16,23–25,27–31,37–40,48,51], were stool tests and anal swabs regularly collected from subjects with or without GIS (Tables 2 and 3). A histological study of intestinal biopsies was performed in one study [30]. The test for the detection of SARS-CoV-2 viral RNA in gastrointestinal samples mostly corresponded to reverse transcription polymerase reaction for amplification of the main genes such as frame1ab ORF1ab, RdRp and nucleocapsid proteins (S, E, N) (Table 3).

Six of eight studies detected by RT-PCR antigens of SARS-CoV-2 in the stool or anal swaps with a significative viral load ($>1\times10^6$ cop/μL or threshold cycle *t* value <40). The frequency ranged from 27 to 100% of samples [3,24,35,40–42]. One study not detected significative viral load in two patients [52] and the other only offers qualitative results [30].

The presence of gastrointestinal symptoms (GIS) was not associated with fecal sample viral RNA positivity ($P=0{\cdot}45$) [41].

Table 1. Demographic characteristics and comparison of quality of the studies included in the review

Primary author/year of publication	Country/region	Sex *n* (%) (M: male; F: female)	Age (months; years)	Monitoring period	Design	STROBE
Zhang W *et al*. (2020) [23]	Wuhan, China/Asia	NK	NK	12 December 2019– 3 February 2020	Descriptive	Medium
Kim JY *et al*. (2020) [24]	South Korea/Asia	Patient 1: Chinese F (primary case) Patient 2: Korean M	35–55 years	December 2019–February 2020	Descriptive	Medium
Xu Y *et al*. (2020) [25]	Guangzhou, China/Asia	6/10 (60) M 4/10 (40) F	Pediatric	22 January–20 February 2020	Prospective observational, 1 site	High
Nicastri E *et al*. (2020) [26]	Rome, Italy/Europe	Asymptomatic Italian M1 from Wuhan	20 years	3–22 February 2020	Descriptive, one case	Medium
Sun D *et al*. (2020) [27]	Wuhan, Hubei, China/Asia	6 M 2 F	R: 2 months–15 years	24 January–24 February 2020	Description of a case series	Medium
Lo IL *et al*. (2020) [28]	Macau, China/Asia	3/10 (30) M 7/10 (70) F	x̄: 54 years R: 27-64 years	21 January–16 February 2020	Descriptive	Medium
Ma X *et al*. (2020) [29]	Shandong, China/Asia	Children 6/8 (75) Adults 2/8 (25)	R: 11 months–39 years	NK	Descriptive	Medium
Xiao F *et al*. (2020) [30]	Guangdong, China/Asia	NK	NK	1–14 February 2020	Descriptive	High
Holshue ML *et al*.(2020) [31]	Washington, USA/America	1 M returning from Wuhan on 15 January	35 years	19–20 January 2020	Descriptive 1 case	Medium
Kim ES *et al*. (2020) [32]	Seoul, South Korea/Asia	15/28 (53.6) M 13/28 (46.4) F	x̄: 40 years R: 20–73 years	19 January–17 February 2020	Cohort	High
Wang D *et al*. (2020) [17]	Wuhan, China/Asia	75/138 (54.3) M 63/138 (45.7) F	x̄: 56 years R: 22–92 years	1 January–3 February 2020	Retrospective cohort	High
Phan LT *et al*. (2020) [33]	Wuhan, China/Asia	2 M (father and child) 1 F	65 years (primary case) 27 years (secondary case) Mother NK	17–20 Jan	Cohort (3-family member cluster)	High
Park JY *et al*. (2020) [34]	Seoul, South Korea/Asia	1/5 Girl, contact with mother and uncle z(traveled to Wuhan) confirmed	Girl 10 years	29 January–18 February 2020	Description of a case, NK for the *cluster*	Medium
Hsih WH *et al*. (2020) [35]	Taichung, Taiwan/Asia	17 (40) M 26 (60) F	x̄: 34.0 years R: 3–68 years	20 January–19 February 2020	Cohort	High
Yang X *et al*. (2020) [36]	Wuhan, China/Asia	35 (67) M 17 (33) F	x̄: 51.9 years	24 December 2019–26 January 2020	Retrospective observational of an outbreak	High
Jin X *et al*. (2020) [37]	Zhejiang, China/Asia	37/74 (50) M 37/74 (50) F	x̄: 46.1 ± 14.1 years	17 January 2020–8 February 2020	Retrospective cohort	High
Pan L *et al*. (2020) [38]	Hubei, China/Asia	97/204 (47.5) M 107/204 (52.4) F	x̄: 52.9 years (SD ± 16)	18 January–18 March 2020	Multicenter cross-sectional descriptive	High
Xing YH *et al*. (2020) [39]	Qingdao, Shandong, China/Asia	NK	Pediatric <10 years R: 1–6 years	17 January–10 March 2020	Retrospective Descriptive	High
Pan Y *et al*. (2020) [40]	Beijing, China/Asia	17 NK	NK	NK	Descriptive	Low
Wu Y *et al*. (2020) [41]	Zhuhai, China/Asia	NK	NK	16 January–15 March 2020	Descriptive	Medium
Guan W *et al*. (2020) [42]	China/Asia	58.1 M 41.9 F	x̄: 47 years R: 35–58 years	11 December 2019–31 January 2020	Multicenter cohort	High
Lu X *et al*. (2020) [43]	Wuhan, China/Asia	104/171 (60.8) M 67/171 (39.2) F	Pediatric <10 years x̄: 6.7 years R: 1 day–15 years	28 January–26 February 2020	Cross-sectional descriptive	Medium
Zhang JJ *et al*. (2020) [44]	Wuhan, China/Asia	71/140 (50.7) M 69/140 (49.3) F	x̄: 57 years	16 January–3 February 2020	Retrospective cohort	High
Liu K *et al*. (2020) [45]	Hubei, Wuhan, China/Asia	61/137 (44.5) M 76/137 (55.5) F	x̄: 57 years R: 20–83 years	30 December 2019–24 January 2020	Retrospective cross-sectional descriptive	Medium
Nobel YR *et al*. (2020) [46]	New York-Presbyterian-Columbia, USA/America	145/278 (52) M 133/272 (48) F	R: 18 years- >70 years	10 March–21 March 2020	Case-controls	High
Cholankeril G *et al*. (2020) [47]	California, USA/America	62 (53.4) M	x̄: 50 years R: 35–67 years	4–24 March 2020	Retrospective cross-sectional descriptive	Medium
Luo S *et al*. (2020) [48]	Wuhan, China/Asia	102/183 (56) M 81/183 (44) F	x̄: 53.8 years	1 January–20 February 2020	Retrospective cross-sectional descriptive	Medium
Lescure FX *et al*. (2020) [49]	Paris, France/Europe	3/5 (60) M 2/5 (40) F	R: 30–80 years	24 January–19 February 2020	Cohort	High
Huang C *et al*. (2020) [3]	Wuhan, China/Asia	30/41 (73) M 11/41 (27) F	x̄: 49 years R: 41–58 years	31 December 2019–2 January 2020	Cross-sectional descriptive	Medium
Chen N *et al*. (2020) [16]	Huanan, China/Asia	67/99 (67.8) M 32/99 (32.3) F	55.5 years (SD: ± 13.1)	1–25 January 2020	Retrospective cross-sectional descriptive	Medium
Young BE *et al*. (2020) [50]	Singapore/Asia	9/18 (50) M 9/18 (50) F	x̄: 47 years	23 January–3 February 2020	Descriptive of an outbreak	Medium
Xu XW *et al*. (2020) [51]	Zhejiang, China/Asia	(35, 5) M	x̄: 41 R: 32–52 years	10–26 January 2020	Descriptive	Low
Wölfel R *et al*. (2020) [52]	Munich, Germany/Europe	NK	NK	23–27 January 2020	Descriptive of a cluster	Medium

Age=may be the mean/median (x̄) and range (R) of ages, NK=data not reflected or is not known; STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist guidelines (observational and descriptive cross-sectional studies), H= high; M= medium; L= low.

Table 2. Gastrointestinal symptoms and enteric involvement according to studies included in the review

Primary author/year of publication	Sample (*N*)	GIS frequency (%)	Diarrhea *n* (%)	Nausea/vomiting *n* (%)	Abdominal pain *n* (%)	Several GIS or other *n* (%)	Positive gastrointestinal Samples
Kim JY *et al*. (2020) [24]	2	2/2 (100)	NK	NK	NK	NK	SS[a]
Xu Y *et al*. (2020) [25]	10	3/10 (30)	3/10 (30)	NK	NK	NK	SS[a]
Nicastri E *et al*. (2020) [26]	56	1/56 (1.7)	NK	NK	NK	NK	SS[a]
Sun D *et al*. (2020) [27]	8	8/8 (100)	3/8 (37.5)	NK	NK	5/8 (62.5)	NK[a]
Lo IL *et al*. (2020) [28]	10	10/10 (100)	8/10 (80)	5/10 (50)	NK	NK	SS[a]
Ma X *et al*. (2020) [29]	27	8/27 (29.6)	NK	NK	NK	NK	SS[a]
Holshue ML *et al*. (2020) [31]	1	1/1 (100)	1/1	1/1	1/1	NK	SS[a]
Kim ES *et al*. (2020) [32]	28	5/28 (18)	3/28 (39)	1/28 (3.6)	1/28 (3.6)	NK	SS[a]
Wang D *et al*. 2020 [17]	138	36/138 (26.1)	14/138 (10.1)	14/138 (10.1) 5/138 (3.6)	3/138 (2.2)	NK	NK[a]
Phan LT *et al*. (2020) [33]	28	3/28 (10.7)	1/3 (33.3)	1/3 (33.3)	NK	NK	NK[a]
Hsih WH *et al*. (2020) [35]	43	2/43 (4.6)	5/43 (11.6)	3/43 (7)	3/43 (7)	NK	NK[a]
Jin X *et al*. (2020) [37]	651	74/651 (11.4)	53/651 (8.1)	10/651 (1.5) 11/651(1.6)	NK	3/74 (4)	NK[a]
Pan L *et al*. (2020) [38]	204	103/204 (50.5)	35/103 (34)	4/103 (3.9)	2/103 (1.9)	81 (78.6)	SS[a]
Xing YH *et al*. (2020) [39]	60	3/60 (5)	1/3 (33.3)	NK	1/3 (33.3)	NK	SS[a]
Wu Y *et al*. (2020) [41]	74	23/74 (31)	NK	NK	NK	NK	SS[a]
Guan W *et al*. (2020) [42]	1099	96/1099 (8.7)	41/1099 (3.8)	55/1099 (5)	NK	NK	NK[a]
Lu X *et al*. (2020) [43]	1391	171/1391 (12.3)	15/171 (8.8)	11/171 (6.4)	NK	NK	NK[a]
Zhang JJ *et al*. (2020) [44]	140	55/139 (39.6)	18/139 (12.9)	24/139 (17.3)	8/139 (5.8)	NK	NK[a]
Liu K *et al*. (2020) [45]	137	11/137 (8)	11/137 (8)	NK	NK	NK	NK[a]
Nobel YR *et al*. (2020) [46]	278	97/278 (34.8)	56/278 (20.1)	63/278 (22.6)	NK	NK	NK[a]
Cholankeril G *et al*. (2020) [47]	116	37/116 (31.9)	12/116 (10.3)	12/116 (10.3)	NK	5/116 (4.3)	NK[a]
Luo S *et al*. (2020) [48]	1141	183/1141 (16)	68/1141 (5.9)	134/1141 (11.7) 119/1141 (10.4)	45/1141 (3.9)	16/1141 (9)	NK[a]
Huang C *et al*. (2020) [3]	41	1/40 (3)	1/40 (3)	NK	NK	NK	NK[a]
Chen N *et al*. (2020) [16]	99	2/99 (2)	1/99 (1)	NK	NK	NK	NK[a]
Young BE *et al*. (2020) [50]	18	4/18 (22.2)	3/18 (16.6)	3/18 (16.6)	NK	NK	SS[a]
Xu XW *et al*. (2020) [51]	62	3/62	3/62 (4.8)	NK	NK	NK	NK[a]
Wölfel R *et al*. (2020) [52]	9	2/9 (22.2)	2/9 (22.2)	NK	NK	NK	SS[a]

Frequency of gastrointestinal infection by SARS-CoV-2; gastrointestinal symptomatology or enteric involvement (GIS); gastrointestinal sampling that usually included: stool swab (SS) or histological samples (H).
SARS-CoV, severe acute respiratory syndrome coronavirus-2.
[a]Respiratory samples could include nasal and pharyngeal swabs, bronchoalveolar lavage fluid, sputum or bronchial aspirates (URT and LRT), other serological samples, but in this review, we focused on studies of intestinal samples. NK, data not reflected or datum not known

Six studies detected positive fecal specimen after negativization in respiratory samples [24,29,30,34,39,41]. The frequency ranged from 23.3 (29) to 78% [41]. Notably, one patient had positive fecal samples for 33 days after the respiratory samples became negative, and another was positive for 47 days after the first symptom onset [41].

Histological samples from the stomach, small intestine and colon were screened for the detection and localization of ACE2 receptor cells, and the nucleocapsid of the coronavirus in 1/71 (1.4%) patients, using staining techniques in 2/33 (6%) articles showing abundant ACE2 in the cytoplasm of glandular cells of gastric, duodenal and rectal epithelia (Table 3). The viral nucleocapsid protein of SARS-CoV-2 was visualized in the cytoplasm of gastric, duodenal and rectum glandular epithelial cells of one patient [30].

Discussion

COVID-19 gastrointestinal symptoms

During the 2002 SARS epidemic, diarrhea was reported in 16.7% of cases [53]. In the MERS epidemic, 26% of cases were reported with diarrhea, 21% with nausea-vomiting and 17% with abdominal pain [54].

In the first studies published on COVID-19, conducted in hospital centers in Wuhan (epicenter of the pandemic), nausea or vomiting was observed in 5% and diarrhea in 3.7% of the cases studied [3,17].

Subsequently, many studies have analyzed the occurrence of GIS, showing great variability coinciding with the pooled analysis. Our analysis showed GIS in 16%, diarrhea in 8.1%, nausea-vomiting in 12% and abdominal pain in 4%.

It is well known that the dominant clinical signs of COVID-19 are respiratory symptoms (cough, dyspnea and fever), but, as has been seen in this review, there is a significant percentage of cases with GIS from the time of patient admission (before starting treatment) and that, sometimes, may precede the respiratory symptoms [17,31]. One study showed that up to 3% of cases may have exclusively presented with GIS [38]. The presence of these GIS has not been related to the positivity of viral RNA in stool [41].

On the other hand, studies are showing that the presence of GIS may indicate a higher probability of a severe course [37,42]. A higher percentage of diarrhea was observed in patients with severe disease (5.8%) than in nonsevere disease (3.5%). Guan *et al.* [42] and a significant serious course was found in patients with GIS (22.97%) than in those without GIS (8.12%) $P < 0.001$ [36]. In another study, differences in GIS prevalence between nonsevere disease (37.8%) and severe disease (42%) were not found $P = 0.610$ [44].

Enteric involvement

The finding of an ACE receptor as the entry for SARS-CoV-2 to the cell suggests that human organs with a high level of ACE2 expressions, such as pulmonary alveolar

Table 3. Fecal excretion of viral RNA of severe acute respiratory syndrome coronavirus-2-2 and the possible fecal–oral transmission pathway based on viral load and intestinal cytology according to the experimental studies included in the review

Primary author/year of publication	Laboratory technique (genes tested)	VLAS (log10 copies/μL, log10 cop/swab, qPCR Ct values)	Positive fecal samples n (%)	Positive fecal samples after negativization respiratory samples *n* (%)	Fecal RNA detection range (days)	Histology	Infer fecal–oral transmission
Zhang W *et al*. (2020) [23]	qRT-PCR by HiScript II One Step qRT-PCR	n_1 = 4/15 (27%) Ct: 30.9–31.2 n_2 = Day 1: 5/16 (31%) Day 5: 6/16 (38%) Ct: 17.8–33.8	*n*:1 = 4/15 (27) *n*:2 Day 0: 5/16 (31) Day 5: 6/16 (38)	NK	NK	NK	NK
Kim JY *et al*. (2020) [24]	rRT-PCR (RdRp/E)	500–700 R: 1/27–1/37 × 10^5	0/2 (0)	NK	R: 4–19	NK	NK
Ma X *et al*. (2020) [29]	qRT-PCR; (ORF1ab/N) nucleic acid detection kit (Bio-germ, Shangai, China) histological	NK	NK	8/23 (34,7)	R: 1–35	NK	Family cluster person-person
Xiao F *et al*. (2020) [30]	qRT-PCR; China Disease Control and prevention-standardized quantitative PCR assay. Duodenal-rectal histology by endoscopy histological staining (H&E) and viral staining of the ACE2 receptor, by confocal laser scanning microscopy (Viral nucleocapsid protein staining)	NK	39/73 (53,4)	17/73 (23,3)	R: 3–10	ACE2 stained positive in the cytoplasm of glandular cells of gastric, duodenal, and rectal epithelia Staining of viral nucleocapsid in the same cells R: image 20–100 mm	Propagation of infected cells to uninfected cells
Park JY *et al*. (2020) [34]	RT-PCR (RdRp/E)	NK	1/1 (100)	1/1 (100)	R: 5–17	NK	Family cluster person-person
Xing YH *et al*. (2020) [39]	RT-PCR (ORFab/N)	3/3 (100) Ct value <40	3/3 (100)	3/3 (100)	R: 4–30	NK	Fecal–oral Possible contaminated fomites
Pan Y *et al*. (2020) [40]	rRT-PCR (N)	9/17 (53%) R: 500–1.21 × 10^5	9/17 (53)	NK	R: 3–15	NK	Family cluster person-person
Wu Y *et al*. (2020) [41]	rRT-PCR kit from LifeRiver Ltd. (Nucleocapsid gen (N), membrane gen (E) and RNA dependent RNA polymerase gene (RdRp).	41/74 (55%) Ct: 28,26 ± 11 R: 8–47	41/74 (55)	32/41 (78)	after first symptom $\bar{x}$: 27.9 (SD: ± 10.7) R:0-42 After respiratory negativization $\bar{x}$: 11.2 (SD: ± 9.2) R: 0–33	NK	Possible fecal–oral
Lescure FX *et al*. (2020) [49]	rRT-PCR (RdRp-IP1/E)	6.8–7.4 × 10^5 g stool	2/5 (40)	NK	R: 2–18	NK	Family cluster person-person
Wölfel R *et al*. (2020) [52]	rRT-PCR (ORFab/N) virus cellular isolation	<106; R: 6.76 × 10^5–7.11 × 10^8	8/9 (89)	5/8 (62,5)	R: 5–12	NK	Family cluster

VLAS, viral load in stool or anal swab: value of viral load detected in stool samples or rectal swabs; R, range of days; M, mean number of days; CS, cytological staining of tissue samples; day of detection: day of infection on which SARS-CoV-2 virus is detected in GI samples; Ct, for viral RNA measurements, some authors used cycle threshold (Ct) values to approximately indicate viral load (inversely related to the Ct value). Significant value for SARS-CoV-2 <40.

ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; NK, data not reflected or is not known; rRT-PCR, reverse transcription polymerase reaction; SARS-CoV, severe acute respiratory syndrome coronavirus-2.

epithelial cells and small intestinal enterocytes, are potentially vulnerable and a target for SARS-CoV-2 infection [29,30,55,56].

The binding of SARS-CoV-2 to ACE2 has been shown to have approximately 10–20 times greater affinity than SARS-CoV via S protein, which may provide an explanation of why SARS-CoV-2 has more person-to-person spread compared with SARS-CoV [57,58]. COVID-19 disease can affect, in addition to the respiratory and gastrointestinal tract, various organs such as the kidneys, liver, musculoskeletal, cardiovascular and neurological systems [59,60].

In this review, we found [28,29] articles supporting the above statement that human ACE2 is a receptor for SARS-CoV-2 expressed in gastric, intestinal and colonic cells [61,62].

The possible infection of the gastrointestinal cells was studied in tissue samples from the esophagus, stomach, duodenum and rectum, and although no significant histological alteration was observed, through staining, the presence in the cytoplasm of the cells of the ACE2 receptors and the nucleocapsid of the SARS-CoV-2 was determined. This indicates the possibility of enteric infection [30]. This enteric infection could release virions and cause possible fecal–oral transmission.

Other reports have suggested that if SARS-CoV-2 can actually infect the human intestinal epithelia, it would have significant implications for fecal–oral transmission and the containment of viral propagation [17,42].

Infection of intestinal cells can be expressed with GIS, such as abdominal pain, vomiting and diarrhea, as demonstrated in some studies [32,63].

One study showed that the extension in days of viral RNA elimination in stools had not been related to disease severity [41].

This reinforces the need for future studies on enteric participation and viral excretion of SARS-CoV-2 in stool and for research on whether fecal SARS-CoV-2 RNA levels correlate with disease severity and the presence or absence of GIS [19].

Fecal levels of SARS-CoV-2 viral RNA and possible fecal–oral transmission of infection

The primary transmission pathway is by inhalation of respiratory microdroplets, but there may be other mechanisms such as: conjunctival, one study showed the presence of RNA in conjunctiva [64]; fecal, another study in Singapore showed the presence of virus RNA in samples from an infected patient's toilet [65] and on fomites, the same study detected the virus on many surfaces of the room [65].

In this regard, it has been postulated that the dynamics of SARS-CoV-2 must be determined to study possible fecal transmission, and it is, therefore, important to take simultaneous respiratory and fecal samples to study the kinetics and viral load of SARS-CoV-2. The cycle threshold (Ct) values for viral RNA measurements reflect, in an inversely related manner, the viral load and are suggested by some authors for expression [25,66].

Viral kinetics in infected patients have not yet been fully determined. Viral RNA in COVID-19 has been found in stool in the early and late phases of the disease at a rate, in the most numerous series, of between one-third and one-half of the cases [23,40,41]. Viral RNA may remain positive in stool samples, up to an average of 11.2 days and up to a maximum of 33 days after being negative in respiratory samples, suggesting that the virus could actively replicate in the gastrointestinal tract of the infected patient and that fecal–oral transmission could occur after viral clearance in the respiratory tract [40,41].

One German study found high viral loads in stool and the presence of subgenomic RNA in some patients, indicating the possible viability of the virus, though it could not be cultured in stool [52].

In contrast, another study found no significant value of viral RNA in stool [37]. A study of the pediatric population showed persistent excretion of SARS-CoV-2 in the stool of children between 8 and 20 days after negativization in respiratory samples. This would increase the possibility of the virus being transmitted through contaminated fomites, so there is a need for massive efforts at all levels to prevent the spread of infection between children after reopening daycare centers and schools, as noted in one of the articles discussed in this review [39].

It has been suggested that the prolonged RNA presence of SARS-CoV-2 after negativization in respiratory specimens may be an infectious source of COVID-19 in the community and may represent a threat to public health if eligibility for discharge is based on the current version of the COVID-19 diagnosis and treatment plan [39,67]. Therefore, SARS-CoV-2 RT-PCR measurement in stool would be recommended following the clearance of viral RNA in respiratory specimens from hospitalized or quarantined patients [39,41].

High viral load in elderly patients has been associated not only with the low immunity of the elderly but also with high expression of the ACE2 receptor (the cellular entry receptor for SARS-CoV-2) in older adults, and further studies with a larger sample size are needed to clarify and understand the relationship between viral load and disease severity [68,69].

In histological studies, some authors have suggested that if SARS-CoV-2 can actually infect the human intestinal epithelium, it would have significant implications for fecal–oral transmission and the containment of viral propagation [17,42].

It has also been suggested that further studies are needed to elucidate the exact role of fecal–oral transmission in the spread of SARS-CoV-2 through environmental studies, and studies on viability and infectivity [19,70].

Strengths and limitations

To our knowledge, this is the first systematic review on the prevalence of GIS and enteric involvement of COVID-19 infection, and also includes studies on the excretion and concentration of SARS-CoV-2 virus in biological gastrointestinal samples and on the possibility of fecal–oral transmission of COVID-19. This is possibly the first study conducted in Spain, where the pandemic is having a severe impact. Several electronic databases were searched for our systematic review, the vast majority of references were retrieved, and a large number of studies related to the subject matter at hand were included. Furthermore, since the data analysis was essentially descriptive, no significant bias is expected from our methodological option.

However, we found substantial methodological limitations. The heterogeneity between studies and the novelty of the pandemic health event constituted an established limitation of systematic reviews, and, in this case, the majority of studies being conducted at this time are ongoing and have not yet been published. To minimize potential bias, we attempted to select all studies published to date globally, regardless of sample size.

Despite the limitations of the data in the reviewed articles, the estimates reported here show the frequency of the GIS and that the presence of SARS-CoV-2 viral RNA in the stool could represent a significant burden for the probable fecal–oral transmission of the infection. Further work is needed to update the case definition, studying enteric involvement through the design of prospective observational studies using a sample size representative of the population that allows results to be outsourced.

Conclusion

GIS are common in SARS CoV-2 infection at the time of patient admission, sometimes preceding respiratory symptoms, and sometimes represent the only clinical manifestation. The case definition evolves rapidly as knowledge accumulates, and the definition could be revised, including these considerations. The presence of GIS could predict a poorer course of the disease. In the context of the current pandemic, adequate clinical suspicion may lead to an early diagnosis and treatment of the disease and may hypothetically reduce the frequency of progression to more severe disease.

Infection of the gastrointestinal tract is possible due to the presence of ACE2 receptors, and there may be viral replication with fecal elimination. Studies are required to assess viability and transmissibility. Viral RNA is detected in the stool for a longer time than in the respiratory system. As has been suggested, its detection in fecal samples should be considered as one of the routine diagnostic tests to guide decision making on hospital discharge and the lifting of isolation measures.

It is advisable to design and conduct prospective epidemiological studies at the community level or using a sample size representative of different populations and to substantiate the preliminary findings made in some case studies reported in this systematic literature review. Such studies will make it possible to determine the actual prevalence of GIS and its potential correlation with severity.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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