Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Fecal-oral transmission of SARS-CoV-2: A systematic review of evidence from epidemiological and experimental studies

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Key Words:
COVID-19
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Background: SARS-CoV-2 ribonucleic acid (RNA) has been detected in feces, but RNA is not infectious. This systematic review aims to answer if fecal SARS-CoV-2 is experimentally infectious and if evidence of human fecal-oral SARS-CoV-2 transmission exists.

Methods: On September 19, 2022, we searched PubMed, Embase, Web of Science, medRxiv, and bioRxiv. Biomedical studies inoculating SARS-CoV-2 from feces, rectal, or anal swabs in cells, tissue, organoids, or animals were included. Epidemiological studies of groups differing in exposure to fecal SARS-CoV-2 were included. Risk of bias was assessed using standardized tools. Results were summarized by vote counting, tabulation, and a harvest plot. PROSPERO registration no. CRD42020221719.

Results: A total of 4,874 studies were screened; 26 studies were included; and 13 out of 23 biomedical studies (56.5%) succeeded in infection. Two (66.7%) epidemiological studies found limited evidence suggesting fecal-oral transmission. All studies had concerns about the risk of bias.

Conclusions: It is possible to experimentally infect cell cultures, organoids, and animals with fecal SARS-CoV-2. No strong epidemiologic evidence was found to support human fecal-oral transmission. We advise future research to study fecal infectivity at different time points during infection, apply appropriate controls, use in vivo models, and study fecal exposure as a risk factor of transmission in human populations.

BACKGROUND

The pandemic coronavirus disease 2019 (COVID-19)[1] was caused by the newly emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; family *Coronaviridae*, genus *Betacoronavirus*).[2,3] SARS-CoV-2 is recognized to be transmitted primarily by droplets and aerosols during close contact, and secondarily by fomites.[4]

Yet, as SARS-CoV-2 ribonucleic acid (RNA) has been detected in the feces of COVID-19 patients, a fecal-oral route of transmission is also considered. A meta-analysis of symptomatic COVID-19 patients reported the prevalence of SARS-CoV-2 RNA reliably detected in fecal samples, rectal, or anal swabs to be 47% (95% confidence interval 38-55).[5] The discovery of in-host genomic variability in fecal SARS-CoV-2 RNA suggests ongoing viral evolution depending on active infection of the gastrointestinal tract.[6]

Direct evidence of enterocyte infection is available, as SARS-CoV-2 RNA and particles were discovered in the biopsies of esophageal, gastric, small intestinal, colonic, and rectal tissues from COVID-19 patients.[7–11] However, the evidence of active infection of intestinal cells along with fecal shedding of SARS-CoV-2 RNA does not necessarily imply fecal-oral transmission to be possible. Reverse transcription polymerase chain reaction (RT-PCR) cannot distinguish transmissible virus particles from fragments with no infectious potential.

Experimentally, human small intestinal and colonic organoids and human colon cell lines have also been successfully infected with SARS-CoV-2.[12–14] Further, intranasal, intragastric, and intratracheal inoculation with SARS-CoV-2 of nonhuman primates led to infection of intestinal segments from the stomach to rectum.[15–17]

On this basis, SARS-CoV-2 is now appreciated as an enteric pathogen. During the COVID-19 pandemic, the World Health Organization (WHO) made recommendations for safe sanitation, for example, to limit exposure to feces from infected individuals.[18] Medical societies around the world also advised endoscopy units to

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postpone nonurgent cases and reorganize their workflow to mitigate the risk of virus transmission.[19] Though the fecal-oral route today is not considered a main route of infection,[4] the possibility of fecal-oral transmission has not yet been ruled out.

The aim of this systematic review was to investigate if SARS-CoV-2 has a potential for fecal-oral transmission by answering if any epidemiologic evidence for or against human fecal-oral transmission exists and if fecally shed SARS-CoV-2 is infectious to culture cells, tissues, organoids, or live animals.

METHODS

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines.[20] The methods were prespecified in a protocol available on PROSPERO (registration no. CRD42020221719).[21]

Two reviewers identified studies for inclusion by title and abstract screening and full-text screening and then performed data extraction, risk of bias assessment, and certainty of evidence assessment. These activities were undertaken independently, with each reviewer blinded to the other's decisions. Disagreements were resolved by discussion and consensus. A third reviewer was available for consultation in case of disagreements, though all discrepancies were resolved by consensus.

Literature search

We designed a search strategy using subject headings and free-text words synonymous with or related to "SARS-CoV-2" and "fecal-oral transmission" combined by Boolean operators. It was developed in cooperation with a health care librarian with systematic review expertise. The reproducible search strings are available in Supplementary Appendix 1.

Five electronic databases were searched (platforms in parentheses): Embase (embase.com), PubMed (pubmed.gov), Web of Science Core Collection (webofknowledge.com), bioRxiv (medrxiv.org), and medRxiv (medrxiv.org). Preprint databases were included to minimize the publication bias.

All searches were run on September 19, 2022. Reference lists of the included articles were manually screened to identify additional studies.

The searches were limited to dates of publication ranging from December 31, 2019, onwards. On that day, the WHO China Country Office was for the first time notified of an outbreak of the disease that would later be known as COVID-19.[22] No language or study design limits were applied.

Study records

The search results were exported to EndNote version X9.3.3 (Clarivate Analytics). Duplicates were identified using EndNote's duplicate identification tool. The records were then uploaded to Covidence (Veritas Health Innovation) for study selection and data extraction.

Study selection

Studies fulfilling the following criteria were included:

- Epidemiologic studies of groups differing in exposure to fecally shed SARS-CoV-2. An outcome should be RT-PCR–confirmed SARS-CoV-2 infection.
- Biomedical studies of cell, tissue, organoid, or live animal inoculation with SARS-CoV-2 obtained from human or animal feces, rectal, or anal swabs. The outcome of infection should be identified by use of electron microscopy or change in viral titer by quantitative RT-PCR.

When the results of the outcome of interest were not sufficiently reported, the study was still eligible, and the corresponding authors were contacted via e-mail (maximum 2 e-mail attempts, time limit of clarification was 1 week).

Published and preprint reports were included.

We excluded abstracts, consensus documents, editorials, exercises, features, forums, guidance, highlights, images, insights, interviews, news, opinions, panels, personal views, perspectives, podiums, policies, practices, protocols, seminars, rapid reviews, reviews, symposia, and previous systematic reviews.

We also excluded comments or commentaries, correspondences, rapid and short reports, and letters to the editor presenting no original results.

Reports in languages other than English were excluded, but relevant titles are listed in the results.

Data extraction

Items recorded from the included studies are listed in Supplementary Table S1.

Risk of bias assessment for individual studies

Two different tools were used. The tool for epidemiologic studies was developed with inspiration from tools of the Joanna Briggs Institute to be suited for different study designs.[23] A new tool for biomedical studies was developed. Both are available in Supplementary Appendix 2.

To assess potential outcome-reporting biases, analyses specified in the methods sections of the included studies, and protocols if available, were compared to the outcomes.

The risk of bias assessment did not affect the final inclusion.

Data synthesis

We used vote counting for data summary. Effect estimates were categorized into binary variables (evidence for or against fecal-oral transmission) and reported as proportions. The included studies were tabulated and summarized in a harvest plot.[24]

Certainty of evidence

Certainty of evidence was assessed by a Grading of Recommendations Assessment, Development, and Evaluation–inspired approach.[25] Four levels of certainty were used: high, moderate, low, and very low. Beginning from high certainty, we voted the level a step down when 1 of the following criteria were met: risk of bias assessments (if more than half of the included studies were assessed medium or high); imprecision (if either epidemiologic or biomedical evidence came from only 1 or 2 small studies); inconsistency (if not a large proportion of the included studies, ie, more than 85%, agreed on the risk of fecal-oral transmission); and indirectness (if most outcomes were surrogates for fecal-oral transmission). As publication bias was not possible to estimate because of the binary effect estimate, this was not included.

RESULTS

The systematic search identified 10,315 records (Fig 1). After duplicates removal we screened 4,869 records, from which we reviewed 152 full text documents and finally included 30 reports of 24 studies.[10,14,15,26–52] A list of studies excluded during full-text screening is available in Supplementary Appendix 3 along with the

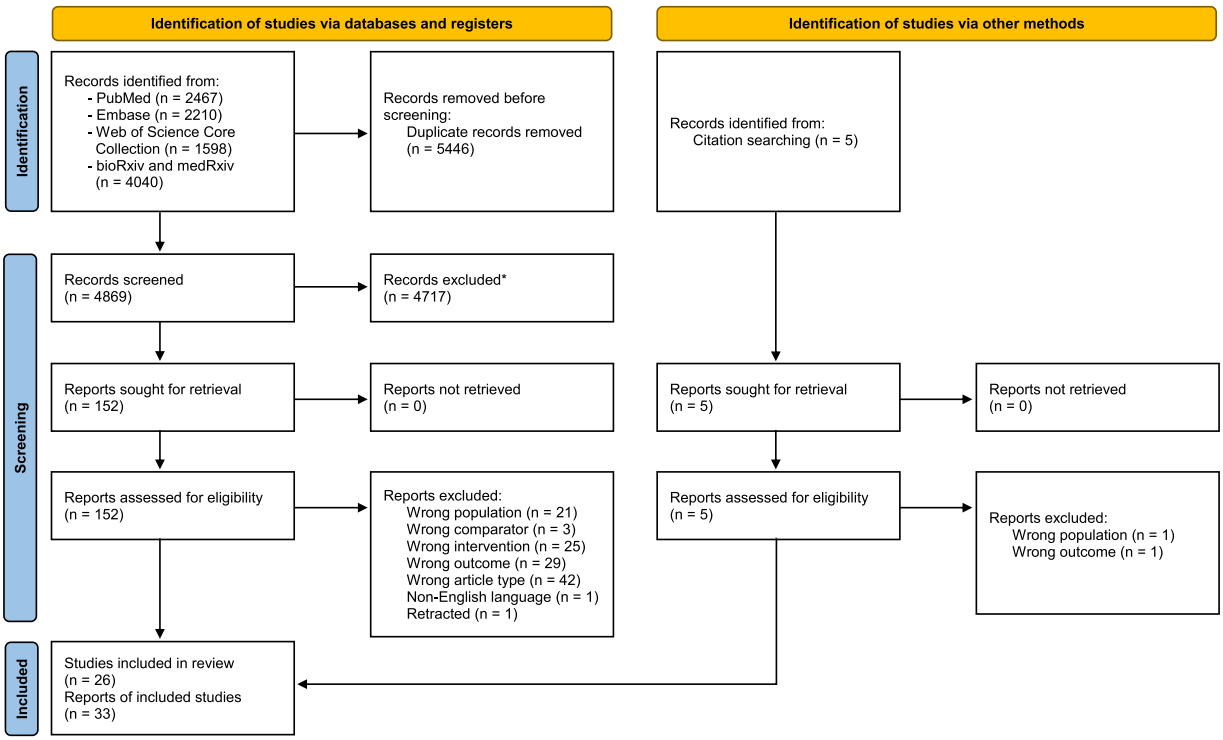

Fig. 1. Flowchart from record identification to study inclusion. *Duplicates were removed using the duplicate identification tool of EndNote version X9.3.3 (Clarivate Analytics) and then manually. Figure setup from Page et al.[20]

reasons for exclusion. One possible eligible record in Chinese was identified, but was excluded because of non-English language.[53] Later, we searched the references of the included reports and other reviews and thus identified 3 reports of 2 additional studies eligible for inclusion.[54–56]

Across all study designs, 15 of 26 included studies (57.7%) provided any evidence favoring possible fecal-oral transmission of SARS-CoV-2.

Epidemiologic studies of fecal-oral SARS-CoV-2 transmission

Three epidemiologic studies were eligible for inclusion (Table 1).

In 2 out of 3 studies (66.7%), the authors concluded fecal-oral SARS-CoV-2 transmission to be a possible explanation of the described spread of infection. Al Mayahi et al described a cluster of COVID-19 patients in a hospital.[26] After a patient developed diarrhea, all 4 health care workers who cleaned up a large spill of loose stool were subsequently infected. Of the remaining health care workers exposed to SARS-CoV-2–positive patients, 2 out of 36 became infected. Kang et al described a COVID-19 dissemination in a high-rise building; all infected persons lived in flats connected by dried-out traps to a shared drainage pipe.[40] In all flats not connected to this drainage pipe, no one became infected.

In 1 study, the authors describe the risk of infection from wastewater as minimal. Isanovic et al followed a group of wastewater treatment plant workers for 6 months, testing them for SARS-CoV-2 every 2 weeks.[38] They found no significant difference between the worker case rate and that of the surrounding population.

Biomedical studies of fecal-oral SARS-CoV-2 transmission

23 studies were eligible for inclusion, inoculating cells, tissues, organoids, or animals in vivo with fecal SARS-CoV-2 (Table 2).

In 13 out of 23 studies (56.5%), SARS-CoV-2 from at least 1 fecal sample or rectal swab succeeded in infection (20 of 145 samples, 13.8%).

The most frequent method was inoculation of different Vero lineage cells (18 studies); using this method, 10 studies succeeded in infection (16 out of 112 samples, 14.3%). The most frequent sample type was feces (18 studies); using this sample type, 11 studies succeeded in infection (18 out of 113 samples, 15.9%). 18 studies used human samples for inoculation, with 9 of these (50.0%) succeeding infection (15 out of 126 samples, 11.9%).

Only the studies by Kim et al and Pedersen et al included infections of cell cultures from other than the Vero-lineage; in total, 25 inoculations of CaCo-2 cells led to zero infections.[41,46] Zhou et al successfully made the only inoculation of organoids.[14] Two studies made inoculations in vivo; Jeong et al used live ferrets with a successful outcome[39]; Wurtzer et al inoculated live golden Syrian hamsters unsuccessfully.[49]

Four studies mentioned doing replicates, all of which had the same outcome for every individual sample.[15,39,46,49]

Risk of bias assessment

There were concerns about the overall risk of bias for all included studies (26/26), with 15 of these assessed as having a high risk of bias (Supplementary Table S2 and Supplementary Appendix 2).

In all included studies the outcomes reported in methods and results were without major discrepancies. All studies were reported in peer-reviewed journals, though some were identified also in preprint reports.[28,37,43,45,54]

For a combined overview of the reviewed studies providing evidence for and against the possibility of fecal-oral transmission of SARS-CoV-2, see Figure 2.[24] The concern of risk of bias seems to be equally distributed between evidence for and against fecal-oral SARS-CoV-2 transmission.

Table 1
Characteristics of the included epidemiologic studies

Source	Publication date*	Site (city or province, country)	Study design	Exposed		Unexposed		Group definition	Group comparability	Support of fecal-oral hypothesis†	Risk of bias
				Infected	Uninfected	Infected	Uninfected				
Al Mayahi ZK et al[26]	05/15/2021	Rustaq, Oman	Case series	4	0	34	2	Exposed group cleaned up diarrhea from a COVID-19-positive patient.	Health workers in the same hospital ward	Yes	Medium
Isanovic M et al[58]	07/04/2022	Columbia, SC, USA	Cohort study	1	42	10,915	352,799	Exposed group worked at a wastewater treatment plant having ½ 12 h daily on facility grounds.	Same area	No	Medium
Kang M et al[40]	09/01/2020	Guangzhou, China	Cross-sectional	4	57	0	136	Exposed group lived vertically aligned to the index patient in flats connected by bathroom drainage pipes.	Same apartment complex with similar restrictive measures	Yes	Medium

*MM/DD/YYYY.
†Conclusion by the study authors.

Certainty of evidence

The certainty of the evidence for fecal-oral transmission of SARS-CoV-2 was judged low. The evidence was downgraded 1 step each for risk of bias assessment, inconsistency, and indirectness.

DISCUSSION

This study set out to review the available biomedical and epidemiologic evidence of fecal-oral SARS-CoV-2 transmission. We found that fecally shed SARS-CoV-2 can infect culture cells and organoids in vitro and ferrets and golden Syrian hamsters in vivo, and that fecal-oral transmission is backed up by limited epidemiologic evidence (Tables 1 and 2).

The epidemiologic evidence for fecal-oral SARS-CoV-2 transmission can be described as circumstantial. Kang et al reported probable fecal-oral transmission of SARS-CoV-2 by bioaerosols through a drainage pipe in a high-rise building in China.[40] Although the authors described the circumstances in great detail, including tracer gas testing, the possibility of other routes of transmission still exists. As a study reported isolation of infectious virus from plastic and steel surfaces for up to 72 hours,[57] undetected fomite transmission might still be rendered probable. The retrospective study design further implies the risk of unreported meetings between the affected families.

Al Mayahi et al reported how a group of health care workers with high exposure to feces from COVID-19 patients were infected. The authors emphasize other routes of infection to be equally possible. As Kang et al and Al Mayahi et al only described situations and did not apply any statistics, it is difficult to quantitate the outcome and estimate the likelihood of observing the reported situation if fecal-oral SARS-CoV-2 transmission is not possible. Also considering our risk of bias assessment of both studies to be medium, we find it difficult to conclude anything from these 2 studies.

Isanovic et al reported how there was no difference between SARS-CoV-2 infection in a wastewater treatment plant and the surrounding community. A recent study found a higher prevalence of certain gastrointestinal pathogens in fecal samples from wastewater treatment plant workers than from the more distant surrounding population.[58] This may be explained by the increased exposure to droplets, hand-to-mouth contact, or inhalation of aerosols. Infectious SARS-CoV-2 has been isolated from aerosols for up to 3 hours, which renders the fecal bioaerosol transmission hypothesis possible.[57] In their study, Isanovic et al detected a fecal indicator virus in all air samples collected on the treatment plant grounds, but no SARS-CoV-2 RNA.

Most of the included biomedical studies used Vero E6 cell assays or other Vero cell lines to evaluate infectivity. Vero E6 cells have been used for SARS-CoV-1 research by many laboratories.[59] Accordingly, the cell line was a natural choice for SARS-CoV-2 research, although it has been suggested that Vero E6 cells are less susceptible to SARS-CoV-2 infection than enteroids[14] or human primary airway epithelial cells.[2]

It is interesting how Jeong et al succeeded in infection of ferrets but were not able to determine the outcome from infection of Vero cells because of cell toxicity.[39] Ferrets and golden Syrian hamsters have been found to be excellent animal models of SARS-CoV-2 infection and transmission.[60,61] Generally, animals may be more relevant models of disease transmission between humans compared to cell cultures.

The quantity of SARS-CoV-2 in the swabs or samples is most likely important for the probability of isolation. A significant negative relation between the RT-PCR cycle threshold (Ct) value and culture positivity of Vero E6 cells has been discovered elsewhere,[62] and 2 studies found that only respiratory samples with an RT-PCR Ct value below 24 or 30 were capable of successfully infecting Vero CCL-81 cells[63] or Vero E6 cells,[56] respectively. The relation between Ct value and culture positivity is supported by our findings, as only 2 included studies succeeded in infection with a sample Ct greater than 30.[14,36] In this context, it should

Table 2
Characteristics of the included biomedical studies

Source	Publication date*	Site (city or province, country)	Sample type	Sample origin	Sample note	Donors	Samples	Sample Ct-range	Inoculation test system	Outcome measure	Infection success rate†	Risk of bias
Albert S et al[27,28]	05/11/2021	Valencia, Spain	Feces	Human	In Sigma Virocult at 4 °C for a maximum of 24 h before processing	5	8	31.8-41.3	Cells, Vero E6	qPCR	0/8	Medium
Audsley JM et al[29]	05/10/2021	Melbourne, Australia	Feces	Human	Placed in viral transport medium	1	2	23-36	Cells, Vero/ hSLAM	qPCR	1/2	Medium
Bailie CR et al[30]	02/10/2021	Melbourne, Australia	Rectal swab	Human	N/A	2	2	37-38	Cells, Vero/ hSLAM	qPCR, TEM	0/2	High
Barroso-Arévalo S et al[31]	07/15/2021	Madrid, Spain	Rectal swab	Dog	Placed in viral transport medium	1	1	26.34	Cells, Vero E6	qPCR	1/1	High
Binder RA et al[32]	09/09/2020	Durham, NC, USA	Rectal swab	Human	Self-collected. Placed in viral transport medium	3	3	33.3-38.6	Cells, Vero E6	qPCR	0/3	Medium
Cerrada-Romero C al[33]	05/05/2022	Madrid, Spain	Feces	Human	N/A	27	31	24.3-39.6	Cells, Vero E6	qPCR	0/31	High
Das Adhikari U et al[10]	07/10/2021	Boston, MA, USA	Feces	Human	N/A	5	5	4.5×10^3-7.2×10^7 (RNA copies/mL)	Cells, Vero E6	qPCR	3/5	Medium
Dergham J et al[34]	06/18/2021	Marseilles, France	Feces	Human	Stored at −80 °C	1	2	16.22-17.29	Cells, Vero E6	qPCR	2/2†	Medium
Fumian TM et al[35]	01/09/2021	Rio de Janeiro, Brazil	Feces	Human	Stored at −80 °C for up to 7 months	4	4	29.8-32.4	Cells, Vero E6	qPCR	0/4	High
Gortázar C et al[36,37]	06/12/2021	Castilla-La Mancha, Spain	Rectal swab	Ferret	N/A	1	1	34.5	Cells, Vero E6	qPCR	1/1	High
Jeong HW et al[39]	07/23/2020	Cheongju, Korea	Feces	Human	N/A	1	1	2.2 ($\log_{10}$ RNA copies/mL)	In vivo, Ferrets	qPCR	1/1‡	High
Jiao, L et al[15]	12/08/2020	Yunnan, China	Feces	Rhesus monkey	N/A	N/A	N/A	N/A	Cells, Vero E6	qPCR, TEM	1/N/A	High
Kim JM et al[41]	05/08/2020	N/A, Korea	Feces	Human	N/A	8	13	27-27,310 (RNA copies/μL)	Cells, CaCo-2	qPCR	0/13	High
McAloose D et al[42–45]	10/13/2020	Bronx, NY, USA	Feces	Tiger, lion§	Stored at −80 °C	7	14	22.3-36.3**	Cells, Vero (CCL-81) Vero 76 Vero E6	qPCR	2/14	High
Pedersen RM et al[46]	11/09/2021	Odense, Denmark	Rectal swab	Human	N/A	N/A	12, 13††	25-39‡‡	Cells, CaCo-2 Vero E6	qPCR	0/12 0/13	Medium
Wang W et al[47]	03/11/2020	Hubei, Shandong and Beijing, China	Feces	Human	N/A	N/A	4	22.3-38.4§§	N/A	TEM	2/4	High
Wölfel R et al[48]	04/01/2020	Munich, Germany	Feces	Human	Shipped in native conditions	4	13	3.5-7.7 ($\log_{10}$ RNA copies/g)‡‡	Cells, Vero E6	qPCR	0/13	High
Wurtzer S et al[49]	08/15/2022	Ivry-sur-Seine, France	Feces	Golden Syrian hamster	Inoculation within 30 min of collection	2	2	4×10^6-6×10^7 (RNA copies/mg)	In vivo, Golden Syrian Hamster	qPCR	0/2	Medium
Xiao F et al[50]	05/18/2020	Guangzhou, China	Feces	Human	N/A	1	N/A	23.34	Cells, Vero E6	TEM	1/1	High
Yao H et al[54,55]	10/29/2020	Zhejiang, China	Feces	Human	Viral isolation within 4 h from sampling	3	3	< 28	Cells, Vero (CCL-81)	qPCR	3/3	High
Young BE et al[56]	08/28/2020	Singapore	Feces	Human	Shipped in native conditions	7	7	N/A	Cells, Vero E6	qPCR	0/7	High

(*continued on next page*)

Table 2 (*continued*)

Source	Publication date*	Site (city or province, country)	Sample type	Sample origin	Sample note	Donors	Samples	Sample Ct-range	Inoculation test system	Outcome measure	Infection success rate†	Risk of bias
Zhang Y et al[51,52]	11/25/2020	Beijing, China	Feces	Human	N/A	1/1	1/1	24	Cells, Vero	TEM	1/1	High
Zhou J et al[14]	05/13/2020	Hong Kong, China	Feces	Human	From an archive	1	1	33.6	Organoids, Human enteric	qPCR	1/1	Medium

Ct-range, cycle threshold-range; *N/A*, not available; *qPCR*, reverse transcription quantitative polymerase chain reaction; *TEM*, transmission electron microscopy.

* MM/DD/YYYY. Publication date of the first peer-reviewed report.

† Number of samples resulting in infection out of the total samples inoculated. Except for the lack of clarity in Dergham et al,[34] in all studies doing replicates, all replicates of each sample had the same outcome.

‡ Data from correspondence with the study authors.

§ 1 Malayan tiger (2 samples), 3 Amur tigers (6 samples), and 3 African lions (6 samples).

** Only available for 7 of 14 samples.

†† 12 samples inoculating Caco-2 cells and 13 samples inoculating Vero E6 cells. Not clear if some of these are the same samples.

‡‡ Read from the figure.

§§ Range for all 44 positive fecal samples. Only 4 samples with low Ct were used for inoculation.

Fig. 2. Harvest plot of the included studies. A combined overview of the evidence of fecal-oral SARS-CoV-2 transmission from all included studies. Inspired by Ogilvie et al.[24] Left panel: either biomedical studies with successful infection of cells, tissues, organoids, or animals by fecal SARS-CoV-2 (success rate ≥ $1/n$) or epidemiologic studies concluding their results are supporting the hypothesis of fecal-oral SARS-CoV-2 transmission. Right panel: the opposite.

be noted that Ct values of different samples from COVID-19 patients vary over the course of the disease.[64]

Studies of respiratory samples have found isolation of infectious SARS-CoV-2 to be possible for significantly fewer days than SARS-CoV-2 RNA detection.[65] This supports the hypothesis that the timing of sampling may be important. Because of inconsistent reporting of symptom onset and no systematic sampling across the included studies, no specific conclusions can be made on the temporal distribution of the probability of detecting infectious fecal SARS-CoV-2.

This study had several strengths. A wide search strategy helped in retrieving a high number of results. Searching several databases including preprint servers limited the risk of publication bias. To further limit the bias, 2 researchers did all the screening, data extraction, bias assessments, and certainty of evidence assessment separately and blinded to each other's decisions. Our results contribute to the existing knowledge by giving a comprehensive summary of the current research on SARS-CoV-2 fecal-oral transmission.

Also, this study had some limitations. First, the evidence presented in this manuscript neither excludes nor proves fecal-oral transmission of SARS-CoV-2 between humans. However, several biomedical studies have confirmed the hypothetical possibility. Second, in the screening process, the reviewers were not blinded to journal titles, study authors, or their institutions. Third, vote counting provides no information on the magnitude of effects, and it does not account for differences in the relative sizes of included studies.[66] However, vote counting was assessed the best method, as there was no consistent effect measure. Fourth, to increase the certainty in diagnosis, only epidemiologic studies of RT-PCR-confirmed participants were included. This resulted in exclusion of studies based on seropositivity as a marker of previous infection (see Supplementary Appendix 3). It may therefore be possible to locate more epidemiologic evidence, although the risk of bias from reduced diagnostic accuracy will then be present.

Implications for practice and policy

Generally, findings from this systematic review support the biomedical hypothesis of infectious SARS-CoV-2 in feces but found only limited circumstantial evidence of documented fecal-oral transmission between humans. As the overall certainty of evidence was judged low,

we emphasize that fecal-oral SARS-CoV-2 transmission between humans is still only hypothetical. It could be discussed if, for example, the WHO recommendations on sanitation[18] and the medical society recommendations on endoscopy workflow[19] are necessary as precautionary measures to fecal-oral transmission.

Implications for research

There are a couple of important questions that should be addressed in future research:

1) We propose larger and more systematic cell culture infection studies assessing the timeframe from symptom onset to when it is possible to isolate infectious SARS-CoV-2 from feces.
2) As all but 2 included cell inoculation studies did not apply positive and negative controls, we emphasize the necessity of appropriate controls to ensure the reliability of cell assays.
3) We recommend testing the fecal-oral hypothesis by natural exposure of animals to feces from infected individuals. This will provide stronger evidence on whether exposure to feces containing infectious viral particles actually may lead to infection of organisms more complex than culture cells.
4) Also, we propose controlled epidemiologic studies examining shared toilets and direct exposure to human feces as possible risk factors of SARS-CoV-2 transmission. This may provide the most transferable knowledge on the effect of efforts taken to limit fecal-oral transmission.

CONCLUSIONS

In conclusion, the present systematic review has found 3 epidemiologic studies, of which 2 studies based on circumstantial evidence supported the hypothesis of possible fecal-oral SARS-CoV-2 transmission and 1 cohort study did not. Further, we found 23 studies experimentally inoculating culture cells, organoids, and live ferrets and hamsters with fecally shed SARS-CoV-2, leading to successful infection in 13 studies (56.5%).

Although this systematic review found fecal SARS-CoV-2 to be infectious, the study found no strong direct evidence for fecal-oral transmission of SARS-CoV-2 between humans. Because of comprehensive concerns of the risk of bias in the included studies and the majority of evidence being experimental infection of culture cells, this study, only with low certainty, supports that SARS-CoV-2 has a potential for fecal-oral transmission between humans.

ETHICAL APPROVAL STATEMENT

Ethical approval was not required because this study retrieved and synthesized data from already published studies.

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APPENDIX A. SUPPORTING INFORMATION

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ajic.2023.04.170.

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