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SARS-CoV-2: Potential feco-oral transmission and implications on the spread and severity of COVID-19 in Venezuela. Mini-review.

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Key words: SARS-CoV-2; COVID-19; transmission; Venezuela.

Abstract. The recognized human-to-human transmission of SARS-CoV-2 is through respiratory droplets and contact with contaminated surfaces. However, the high transmissibility of the virus and the pattern of symptoms of COVID-19 suggest the likelihood of other forms of spread. Increasing evidence suggests that SARS-CoV-2 could be transmitted by the feco-oral route. SARS-CoV-2 is known to infect gastrointestinal epithelial cells and a significant number of infected people have gastrointestinal symptoms. Viable viruses, viral RNA, and prolonged shedding of viral RNA have been detected in the feces of COVID-19 patients. The virus has been found in sewage and surface waters of several countries. The possible feco-oral transmission of SARS-CoV-2 could be significant in low-income countries. High poverty levels and the collapse of health and other public services might increase the risk of Venezuelans to suffer a more devastating impact from COVID-19 than other populations. In conclusion, the feco-oral transmission of SARS-CoV-2 has not been demonstrated. However, it is conceivable and the impact of COVID-19 could be high in low-income countries, especially in Venezuela due to its humanitarian crisis. The lack of information on the viability and infectivity of the virus in wastewaters and surface waters and the risk of transmission of the infection are important gaps in knowledge that deserve further investigation.

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SARS-CoV-2: Potencial transmisión feco-oral e implicaciones en la propagación y gravedad del COVID-19 en Venezuela. Mini-revisión.

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Palabras clave: SARS-CoV-2; COVID-19; transmisión; Venezuela.

Resumen. La transmisión reconocida del SARS-CoV-2 de persona a persona es a través de gotitas respiratorias y contacto con superficies contaminadas. Sin embargo, la gran transmisibilidad del virus y el modelo de síntomas del COVID-19 hacen pensar en la probabilidad de otras formas de propagación. Evidencias crecientes sugieren que SARS-CoV-2 podría transmitirse por vía feco-oral. Se sabe que SARS-CoV-2 infecta las células epiteliales gastrointestinales y un número significativo de personas infectadas tienen síntomas gastrointestinales. En las heces de pacientes con COVID-19, se han detectado virus viables, ARN viral y la eliminación prolongada de ARN viral. Se ha detectado el virus en las aguas residuales y superficiales de varios países. La posible transmisión feco-oral del SARS-CoV-2 podría ser significativa en países de bajos ingresos. Los altos niveles de pobreza y el colapso del sistema de salud y de otros servicios públicos podrían aumentar el riesgo de los venezolanos de sufrir un impacto más devastador de la COVID-19 que otras poblaciones. En conclusión, la transmisión feco-oral de SARS-CoV-2 no se ha demostrado. Sin embargo, es concebible y el impacto de la COVID-19 podría ser alto en países de bajos ingresos, especialmente en Venezuela debido a su crisis humanitaria. La falta de información sobre la viabilidad e infectividad del virus en aguas residuales y superficiales y el riesgo de transmisión de la infección son brechas importantes en el conocimiento que necesitan una mayor investigación.

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INTRODUCTION

The novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) leading to Coronavirus Disease 2019 (COVID-19), first appeared in Wuhan, China (1) and headed to a pandemic, with meaningful worldwide effect: ~149,6 million cases and ~3,1 million deaths as of April 29, 2021 (2). The recognized human-to-human transmission of SARS-CoV-2 is through respiratory droplets from infected individuals, mainly during close contact (3) and to a lesser extent by touching contaminated surfaces before contacting mucous membranes (4). However, the sources and transmission routes of the virus remain unclear; its strong infectivity, transmissibility, and the symptomatic features of Covid-19 suggest the likelihood of other forms of spread. SARS-CoV-2 might be transmitted through the feco-oral route since there are proofs of fecal contamination of SAR-CoV and MERS-CoV and their capacities to survive in stools (5). Currently, feco-oral transmission has not been demonstrated. However, increasing evidence suggests this mode of spread (6-14). The aim of this paper is to highlight the most important evidences that support the potential feco-oral transmission of SARS-CoV-2 and the implications on COVID-19 in Venezuela.

SARS-CoV-2 characterization

Coronaviruses belong to the *Coronaviridae* family. Like other coronaviruses, SARS-CoV-2 is a membrane-enveloped, single-stranded, positive-sense RNA virus with nucleocapsid, and a crown-like appearance under an electron microscope. due to the presence of spike glycoproteins anchored to the envelope. SARS-CoV-2 belongs to the genus *Betacoronavirus*, and its RNA genome is 29,891 nucleotides in size, encoding 9,860 amino acids (15). The virus uses the envelope-anchored spike glycoproteins to mediate angiotensin-converting enzyme 2 (ACE2) receptor binding and entry into host cells. It's these receptors that act as docking sites for the spike proteins of SARS-CoV-2 to bind to, allowing the viral and cellular membranes to fuse. Then, the virus integrates its RNA into the cell's own replication machinery, facilitating its propagation. SARS-CoV-2 is then able to proliferate throughout the body, creating immune responses, and causing the person to become infected (16).

ACE2s are membrane glycoproteins, highly expressed in organs like lungs, arteries, kidneys, and heart, but in the human body their highest expression is observed in enterocytes, being the intestine the organ most affected by SARS-CoV-2 infections. ACE2 receptors expression patterns in these organs dictate the virus tissue tropism and correlated with manifestations of illness in humans (17).

Coronaviruses are common in nature and may infect a wide range of animals and humans, producing respiratory, gastrointestinal, and neurological diseases (18). Generally, they are responsible of a wide range of common flu and infections of the upper respiratory tract. Most human emerging infectious diseases are of animal origin and result from interspecies transmission. SARS-CoV-2 is initiated by a zoonotic transmission, likely from bats, and spreads rapidly among humans. The pangolin was suggested as a potential intermediate host for the virus (19).

Evidences suggesting potential feco-oral transmission of SARS-CoV-2

Gastrointestinal manifestations

A significant number of persons infected with SARS-CoV-2 have gastrointestinal symptoms. In a group of 1,099 hospital patients with COVID-19 in China, 3.8% had diarrhea and 5% were affected by nausea or vomiting (6). Similarly, 103/204 (50.5%) hospital patients with the disease had digestive manifestations as their main complaint (7).

Both viable SARS-CoV-2 and viral RNA, and extended fecal shedding of viral RNA have been found in the feces of patients with COVID-19. SARS-CoV-2 RNA was identified in the stools of 81.8% cases, even with a negative throat swab result (8), and in asymptomatic individuals (9). In a systematic review with meta-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 symptoms onset. The analyses revealed a pooled positive proportion of 51.8%. From 73 hospitalized patients with SARS-CoV-2 infection, 39 (53.4%) tested positive for viral RNA in stool specimens and 17(43.6%) remained positive after becoming negative in respiratory samples. Positive fecal samples for SARS-CoV-2 of 282/443 (64%) patients remained positive for a mean of 12.5 days, up to 33 days after respiratory samples became negative. Viable SARS-CoV-2 was found in 6/17 (35%) patients (10). In a study from China, the median duration of the virus in stool was 22 days, as compared to 18 days in respiratory airways and 16 days in serum samples (11). Patients with COVID-19 were tested by RT-PCR: 32% (126/398) of pharyngeal swabs and 29% (44/153) of fecal samples tested positive. Live viruses were detected, by electron microscopy, in four SARS-CoV-2 positive fecal specimens from two patients without diarrhea (12). SARS-CoV-2 RNA loads could be as high as 10^8 copies per gram of feces (13).

Presence of ACE2 receptor of SARS-CoV-2 in enterocytes

ACE2 receptor of SARS-CoV-2 is present in the gastrointestinal mucosa. Indeed ACE2 mRNA is highly expressed. In clinical specimens, ACE2 stained positive mostly in gastrointestinal epithelial cells (14). Recently, ACE2 expression was mainly observed in enterocytes and other types of cells. However, in the respiratory system, the expression was limited, with none or only low levels of ACE2 protein. The virus can productively infect human gut enterocytes, highlighting the need to further study virus shedding in the gastrointestinal tract, and the possibility of feco-oral transmission (20).

Presence of SARS-CoV-2 in sewage and surface water

SARS-CoV-2 has also been detected in wastewaters and concern regarding the potential for transmission through water have been raised. Molecular detection of the virus has been reported in untreated wastewaters in USA (21), France (22), Spain (23), Italy, England, The Netherlands, Slovenia, Czech Republic, Canada, the United Arab Emirates, India, Australia, Chile (24), Brazil (25), and Ecuador (26). The virus was also found in treated wastewater in France (22), Spain (27) Italy (28), Germany (29), China (30) and Japan (31).

So far, there are only three available investigations addressing the presence of SARS-CoV-2 in surface waters. In Japan, the virus was not detected in river water samples (32). In Italy, viral RNA was found in three rivers (33). In Ecuador, high SARS-CoV-2 RNA loads were detected, during the peak case of COVID-19, in rivers from urban streams where sewage is discharged directly (26).

Inactivation and infectivity of SARS-CoV-2 from sewage and natural water

The inactivation of coronavirus in water strongly depends on the temperature, the levels of organic matter and the presence of competitors germs. Coronavirus surrogates can survive longer in presence of organic matter. However, the subsistence is greatly decreased with temperatures > 20º C. At low temperatures, the virus presents a higher perseverance (34). No studies on the inactivation or removal of SARS-CoV-2 in water are available, probably because of the reported very low survival of the virus in surface water, wastewater, sludge and biosolids at temperatures higher than 20° C, and the higher inactivation rate of coronavirus when compared with others such as enteric viruses (35). However, the virus poses high risks due to its highly infectious nature and resistance to conventional water and wastewater treatment technologies (36).

The survival of SARS-CoV-2 in surface water and wastewater in the warm seasons and in tropical areas might be highly reduced. One study conducted in five nations suggests a relationship between temperature and the distribution of SARS-CoV-2 (37). However, when comparing the global climate and COVID-19 distribution charts, it was concluded that there was no significant association between temperature, humidity, and the virus distribution (38).

The occurrence of SARS-CoV-2 RNA in raw wastewaters has been demonstrated in several countries affected by the pandemic. Nevertheless, the presence and infectivity of the virus in treated wastewaters and surface waters are still very rare. There appear to be only two studies assessing the infectivity of SARS-CoV-2 from sewage and natural water. In one study, raw and treated samples from three wastewater treatment plants (WWTP) and three river samples from Milano, Italy, were tested for SARS-CoV-2 RNA by means of real time RT-PCR and infectivity test on culture cells. Viral RNA was detected in raw, but not in treated wastewaters and rivers. The results were negative for infectivity (33). The presence of the virus in raw and treated sewage and the infectivity in raw sewage from nine WWTP were studied in several cities of Germany. SARS-CoV-2 RNA was detected in the inflow of all WWTP but infectivity was not found in any (29). The authors suggested that sewage does not appear to be an important route of spread. However, they highlight the importance of carrying out further studies given the preliminary nature of the data (29,33).

SARS-CoV-2 presence and infectivity in treated wastewaters and surface waters are still poorly studied. The ability of these enveloped viruses to infect is associated with the preservation of their capsid (39). It has to be determined if the integrity and infectivity of the virus is lost during the process of the infectivity analyses. To ensure the absence of infectivity in sewage and other aqueous matrices, complementary analyses have been suggested to show whether viruses are intact or there are only RNA strands in the tested samples (24). Nonetheless, studies have proposed that the SARS-CoV-2 RNA might be infectious and that the transmission of these RNAs could be a possible transmission pathway of the COVID- 19 (40).

The lack of information on the ability of SARS-CoV-2 to remain viable in wastewaters and in surface waters, and the absence of data about infectivity of detected viruses from environmental samples, are significant gaps in knowledge. Currently, it is not clear whether the potential presence in wastewater effluent and pathways into freshwater elevates the risk of infection and the spread of COVID-19 (41). Contact with sewage or contaminated water cannot be ruled out. It is interesting to note that high SARS-CoV-2 RNA loads were detected during the peak cases of COVID-19 in Ecuador (26) and virus load rose, coinciding with the onset of pandemic cases in Brazil (25). On the other hand, other more resistant and infectious mutations of SARS-CoV-2 may appear (24).

Although the feco-oral transmission of the virus has not been shown, there are reasons to believe in the existence of this route of spread. This potential mode of transmission could be significant, especially in low-income countries where access to clean water, deficient sanitary facilities, and open defecation are frequent. Discharge of raw sewage directly into water bodies is a common practice in the vast majority of these nations. Sewage systems are nonfunctional, incompletely functional, or nonexistent and the performance of WWTP is suboptimal (42). Under these circumstances, waterborne diseases are of great concern for public health. Pathogens can be dispersed via wastewater and they form the basis for environmental contamination and disease transmission. These factors explain why the burden from feco-oral pathogens is so high in these areas. Although water treatment appears to inactivate CoVs (17), a large quantity of the world's wastewater is released into surface waters and a prolonged exposure to contaminated environmental sources may potentially lead to an increase transmission risk (21). Many researchers warn about the possibility of SARS-CoV-2 infection through contact with sewage or contaminated water or with the aerosols generated in the pumping and treatment systems, in toilets flushing and also in faulty connections of floor drains with the building's main sewer pipe (35).

Potential impact of the Venezuelan crisis on Covid-19 pandemic

In the last two decades, the serious political, economic, and social situation in Venezuela has caused an unprecedented increasing humanitarian crisis. The ongoing trend of hyperinflation and food shortages have enhanced poverty and malnutrition rates; 96% of households are in poverty and 79% in extreme indigence (43). The World Food Program reported that 2,3 million (7.9%) of Venezuelans were severely food insecure (44). The malnutrition rate, according to weight and age, in children under 5 years of age, is 8%, much higher than that of Colombia (3.4%), Peru (3.2%) or Chile (0.5%). According to the height-age indicator, 30% of these children suffer from malnutrition (43). Childhood mortality has increased 63% only in 6 years from 2012-2018 (45).

In Venezuela, at least 82% of the population has very occasional water access and the quality is far below WHO standards, and about 70% of the wastewater amount produced is not collected or treated (46). The lack of a basic level of drinking water, environmental sanitation, and an appropriate sanitary infrastructure, as well as the sustained increase in poverty, foster the prevalence, spread, and dynamic epidemiology of infectious agents. According to the World Health Organization, environmental factors can play a role in more than 80% of the main diseases in the world. By 2012, 12,6 million (23%) of deaths worldwide were attributable to the environment (47). The illnesses with the highest environmental contribution include diarrheal diseases (22%), parasitic diseases, and those transmitted by vectors (48). In fact, in the country where the infrastructure and health systems and public services have collapsed, resurgence of controlled diseases such as measles, diphtheria, malaria, yellow fever, tuberculosis, cholera, and multiple outbreaks have been observed in the last two decades (49).

Vaccine-preventable childhood infections have reemerged in Venezuela. The country contributed 68% (5,525/8,091) of the cases of the measles cases and most of the deaths (73/85) reported in the Americas in 2018 (50). The outbreak of diphtheria began in 2016 and up until 2019, 2,726 suspected cases were registered of which 1,612 were confirmed with 280 deaths (51).

Venezuela has become the epicenter of an increasing malaria outbreak. Between 2000 and 2015, a 359% increase in malaria cases was observed. Between 2016 and 2017, there was a rise of 71% of cases (52). For Chagas disease, 16 outbreaks of oral transmission were recorded nationwide between 2007 and 2018 (53).

Dengue frequency has increased more than five-fold between 2010 and 2016; the average incidence was 211 cases per 100,000 inhabitants (54). Six large outbreaks were documented between 2007 and 2016 (55). The epidemics of chikungunya of 2014 and Zika virus of 2015 in Latin America, reached Venezuela. The attack rate of chikungunya at a national level was 7-14%. However, the rate reached 40–50% in populated urban areas. During the outbreak peak, 6,975 cases per 100,000 inhabitants were registered (56). The incidence of Zika virus during its epidemic peak was 2,057 cases per 100,000 persons and a total of 67,294 cases occurred between 2015 and early 2019 (57). The reemergence of vector-borne diseases in the country have represented a great public health problem.

Feco-orally transmitted protists are endemic in Venezuela, and intestinal parasites constitute a serious public health problem that has been perpetuated over time (58, 59). For decades, we have observed rates of parasitic infections with one or more species of 54-92%, and rates of *Ascaris lumbricoides* and *Trichuris trichiura* of 19.8-74.6% and 26.6-82.8%, respectively, in poor communities (60-63). Prevalence rates of over 65% in rural communities have been reported (64). Permanency of the high burden from feco-oral pathogens in these areas is explained by the relationship between social marginality and these agents, especially by the lack of maintenance of the sewer network or its absence in some areas (58,59). Emerging pathogens like *Cryptosporidium* and *Cyclospora cayetanensis* are endemic in the country (65, 66) and an association between these pathogens and low socioeconomic conditions in the area has been observed (63,67).

Intestinal parasitoses are primarily a cause of morbidity rather than mortality, but they decrease resistance to other nosological agents and are potential factors of diarrhea, anemia, and malnutrition that are important causes of mortality. On the other hand, a potential threat to patients with COVID-19 in helminths endemic areas is the systemic immunomodulatory effects of these parasites through protein secretion and by alteration of the intestinal microbiome, that may influence the severity of other infectious diseases. In long term helminth-infected individuals, the immunomodulation of the innate and adaptive immune response is characterized by a deviation to a Th2 pattern cytokines. Helminth infections, that are among the most common infectious diseases in the area, may suppress the essential immune response against intracellular pathogens (68). Studies from Venezuelan Warao natives showed Th2 skewed cytokine profiles that facilitated *Mycobacterium tuberculosis* infection in patients with *Ascaris lumbricoides* (69). *Schistosoma* infection is correlated with increase transmission of HIV and deworming with decrease of viral load and improve of CD4+ counts among HIV-infected individuals were noted (70). Lower levels of INF-γ and higher levels of IL-10 and TGF-β in individuals co-infected with *Plasmodium*, *Ascaris lumbricoides* and hookworms, as compared to those infected only by *Plasmodium,* were observed (71). In long term helminth-infected individuals co-infected with SARS-CoV-2, the Th2 pattern cytokines could enhance viral replication of SARS-CoV-2 and severity of COVID-19 (72). The possible implications of the immune-regulatory role of helminths in humans co-infected by SARS-CoV-2 and its effect on the viral disease outcome must be considered.

The situation of extreme poverty, malnutrition, collapse of the health and other public services, and complex epidemiological situation indicate the great risk of the Venezuelan population to suffer a more devastating impact of COVID-19 than in other regions since this country is the poorest in Latin America and is ranked second in terms of extreme poverty worldwide, only surpassed by Nigeria (43).

In the current scenario of the country, and the increase in the number of SARS-Co-2 infected cases, a significant spread of COVID-19 could be possible in the region and in other countries due to the great diaspora of Venezuelan citizens. This panorama is not consistent with the official low number of SARS-CoV-2 infections and deaths from COVID 19 in the country (194,959 cases and 2,099 deaths as of April 29, 2021) (73) and contrasts with the much higher figures observed in other South American regions with obvious better socioeconomic conditions. Brazil, Argentina, Colombia, Peru, and Chile are the nations with the most cases and deaths in this region (2). In the Venezuelan-Colombian border there is a great deal of population exchange. The Ministry of Health and Social Protection from Colombia reported ~2,8 million cases and 72,725 deaths as of April 29, 2021 (74). This suggest that the official figures from Venezuela appear to underestimate the evidence. The situation in Venezuela is dramatic. The country urgently needs international humanitarian aid to alleviate starvation, and morbidity and mortality from Covid-19 and several other diseases.

In conclusion, feco-oral transmission of SARS-CoV-2 has not been shown. However, it is conceivable and COVID-19 impact might be high in low-income countries, especially in Venezuela due to its humanitarian crisis. The lack of information about the viability and infectivity of SARS-CoV-2 in wastewaters and surface waters and the risk of transmission of the infection are important gaps in knowledge that deserve further investigation.

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