Abou-Seri et al. | Article 7766
J Pure Appl Microbiol. 2022;16(3):1447-1464. doi: 10.22207/JPAM.16.3.51
Received: 15 April 2022 | Accepted: 04 July 2022
Published Online: 19 August 2022

P-ISSN: 0973-7510; E-ISSN: 2581-690X

REVIEW ARTICLE OPEN ACCESS

Investigating the Potential Effects of COVID-19 Pandemic on Intestinal Coccidian Infections

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Abstract

New infectious agents pose a global threat to the healthcare system, and studies are conducted to estimate their health and epidemiological outcomes in the long run. The SARS-CoV-2 virus, which has caused the COVID-19 disease, was formerly assumed to be a respiratory virus; however, it can have serious systemic effects, affecting organs such as the gastrointestinal tract (GIT). Viral RNA was reported in the stool in a subset of patients, indicating another mode of transmission and diagnosis. In COVID-19, prolonged GIT symptoms, especially diarrhea, were associated with reduced diversity and richness of gut microbiota, immunological dysregulation, and delayed viral clearance. Intestinal coccidian parasites are intracellular protozoa that are most typically transmitted to humans by oocysts found in fecally contaminated food and water. Their epidemiological relevance is coupled to opportunistic infections, which cause high morbidity and mortality among immunocompromised individuals. Among immunocompetent people, intestinal coccidia is also involved in acute diarrhea, which is usually self-limiting. Evaluating the available evidence provided an opportunity to carefully consider that; the COVID-19 virus and coccidian protozoan parasites: namely, *Cryptosporidium* spp., *Cyclospora cayetanensis,* and *Isospora belli*, could mutually influence each other from the microbiological, clinical, diagnostic, and elimination aspects. We further systemically highlighted the possible shared pathogenesis mechanisms, transmission routes, clinical manifestations, parasite-driven immune regulation, and intestinal microbiota alteration. Finally, we showed how this might impact developing and developed countries prevention and vaccination strategies. To the best of our knowledge, there is no review that has discussed the reciprocal effect between coccidian parasites and COVID-19 coinfection.

Keywords: COVID-19, Intestinal Coccidiosis, Microbiome, Opportunistic Parasites, Pathogenesis, Severity

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Citation: Abou-Seri HM, Abdalgaber M, Zahran F. Investigating the Potential Effects of COVID-19 Pandemic on Intestinal Coccidian Infections. *J Pure Appl Microbiol.* 2022;16(3):1447-1464. doi: 10.22207/JPAM.16.3.51

INTRODUCTION

Emerging infectious organisms represent a challenge to researchers and the healthcare system, who anticipate their long-term epidemiological and health consequences. Additional risk factors are frequently associated with these agents, leading to a more severe course, and necessitating innovative diagnostic approaches. COVID-19 is one of these newly emerged infectious agents.[1] The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes COVID-19, an acute viral disease of the respiratory system. Coronaviruses are relatively large, enveloped viruses that can infect a broad spectrum of mammals.[2]

More than two billion people suffer from parasitic intestinal infections globally, with disproportionately higher prevalence rates in resource-poor countries.[3] Severe parasitic diseases can be manifested by micronutrient deficiency, anemia, malabsorption syndrome, organ damage, malnutrition such as stunted growth, secondary bacterial sepsis, and even death. Pathology and severe type of clinical presentation are frequently associated with a high intensity of infection, which is one of the critical factors related to the clinical presentation of parasitic infection.[4] Among the most significant contributors to the global parasitic illness burden in intestine are the multicellular and highly complex parasites.[3,5] Intestinal coccidia, in particular, are intracellular protozoa of the intestinal epithelium that belong to the Apicomplexa phylum. They have an asexual and sexual reproductive life cycle, producing resistant parasitic stages known as oocysts that are expelled into the environment, enabling the spread of infection.[6] Their epidemiological significance is linked to opportunistic infections associated with a high rate of mortality and morbidity in HIV/AIDS patients. This parasitic group is involved in acute diarrhea, usually self-limited in immunocompetent.[7] The most common agents involved in coccidiosis are *Cryptosporidium* spp.*, Cyclospora cayetanensis,* and *Cystoisospora belli*, which have a global distribution. However, they are more frequently reported in developing countries, principally tropical and subtropical zones.[8]

As parasites and microorganisms have similar pathogenic mechanisms and could cause similar inflammatory, allergic, and immunological reactions, co-infections may lead to misdiagnosis and deceptive estimates of the actual prevalence of single infectious agents. The overall prognosis of infection can be influenced by such coexistence.[1] COVID- 19 symptoms can be mistaken for a range of other pulmonary symptoms, some of which are parasitic. Similarly, SARS-CoV-2 can produce nausea, diarrhea, vomiting, and abdominal pain, which are all signs of intestinal parasites.[1] Unlikely, severe COVID-19 etiology has been associated with an immune hyperactivation phenomenon[9] similar to that observed in chronic inflammatory conditions, for example, diabetes, obesity, inflammatory bowel disease, and hypertension.[10,11] Lifestyle factors such as a higher standard of living, a high-calorie diet, and physical inactivity, together with reduced prevalence of helminthic infections, have been related to the emergence of chronic inflammatory disorders in high-income countries (HICs), according to the hygiene hypothesis.[12,13] Hence, it might be expected that lack of parasite co-infection in such regions could make COVID-19 more severe.[12-15] However, immunomodulation triggered by the helminthic infection might result in greater vulnerability to some infections, decreased susceptibility to others, varying degrees of allergy, autoimmune and inflammatory disease. It could also account for poor vaccine responses and most likely higher level of tolerance to SARS-CoV-2 infection.[16-18] Accordingly, the need for further studies has been highlighted to directly assess the potential impact of helminth infections on COVID-19 severity.[19]

Since parasites and human microbiota have a complex interaction, thus they are capable of triangulating with the host's responses to bystander antigens, immunological homeostasis, vaccinations, or other non-communicable and communicable diseases.[20,21] Accordingly, COVID-19 severity might be muted by parasitic infections through direct modulation of the immune system along with indirect parasite-driven microbiome balance.[22-24] However, COVID-19 can cause weakening of the immune system, especially with the usage of corticosteroids giving rise to the risk of co-infection with coccidian parasites. Currently, difficulty in diagnosis occurs due to overlapping of symptoms and the overall attention given to COVID-19 symptoms neglecting the possibility

of other associations. Clinicians should suspect co-infection in case of occurrence of new onset of symptoms or involvement of multiorgan system.[25] Moreover, Abdel-Hamed et al.,[26] found parasitic infections among COVID-19 patients with a higher prevalence for *Toxoplasma gondii* and *Cryptosporidium* spp. indicating the importance of rolling out opportunistic pathogens in COVID-19 infected patients.

Reviewing the available data provides an opportunity to consider that COVID-19 virus and intestinal coccidian parasites, namely, *Cryptosporidium* spp., *Cyclospora cayetanensis,* and *Isospora belli*, can mutually influence each other from the microbiological, clinical, diagnostic, and elimination aspects (Figure 1). As a result, the goal of the current review is to summarize and consolidate various data sources to offer an overview of knowledge gaps and theories regarding the pathogenesis of COVID-19 infection, which is still causing severe effects on the global economy and health of a population.

COVID-19 and Intestinal Coccidian Parasites: Do they Share a Key Mechanism of Pathogenesis?

Cryptosporidium spp. causes cryptosporidiosis, an intestinal infection characterized by profuse watery diarrhea. Over thirty *Cryptosporidium* spp. have been identified, with some being host-specific and others being more promiscuous within the context of host infectivity.[27] Human infections are usually associated with *Cryptosporidium parvum (C. parvum)* and *Cryptosporidium hominis (C. hominis). C. hominis* is mainly linked to human infections. Unlikely, *C. parvum* is linked to animal infections, particularly in young ruminants.[27] Cryptosporidiosis is limited to the gastrointestinal epithelial lining, causing minimal invasion and penetration through mucosal layers, and are autophagic. To compensate for the lack of some metabolic systems, *Cryptosporidium* spp. interacts with its host.[28] On the *Cryptosporidium* spp. site, multiple proteins have been identified localized on the surface of sporozoite, guiding the host-parasite interactions. They are implicated in initial attachment and further invading infectious sporozoites to host cells. These proteins include mucin-like glycoproteins GP900, circumsporozoite-like protein (CSL), GP60 (proteolytically cleaved into GP40/15 mature glycopeptides), mucins (CpMuc4, CpMuc5), thrombospondin related adhesive protein (TRAP-C1), P23, CP47, Cpa135, CPS-500, and C-type lectin (CpClec).[29]

Cyclospora cayetanensis (*C. cayetanensis*) is the etiological agent of Cyclosporiasis, a food, and water-borne intestinal parasitic disease. *C. cayetanensis* has only been isolated from humans.

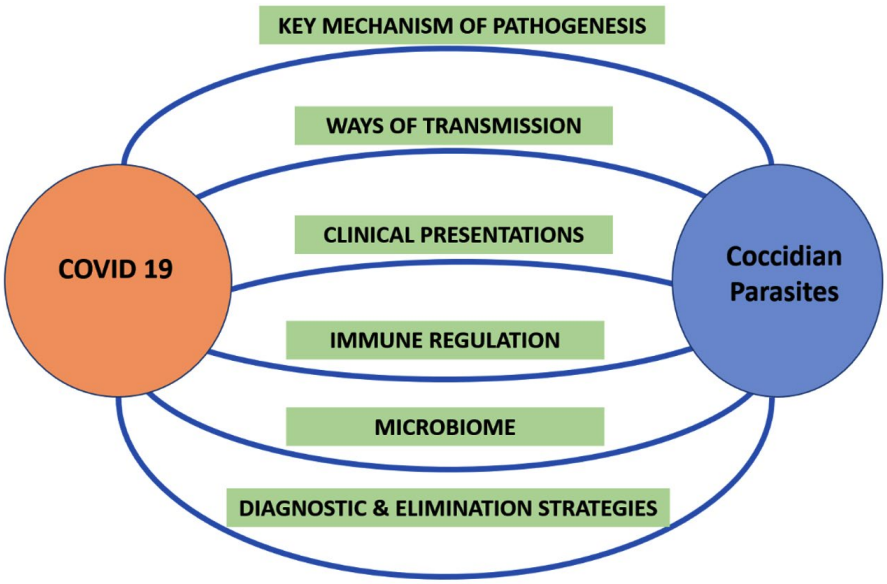

Figure 1. What might COVID-19 and Intestinal Coccidian parasites share in common?

Non-human primates have been known for the isolation of other *Cyclospora* species; however, genetically similar isolates to *C. cayetanensis* have been described from macaques and captive chimps in Europe.[30,31] Invasion and multiplication of *Cyclospora* in enterocytes damage the epithelium of the small intestinal, causing brush border disruption, blunting and atrophy of the intestinal villous, and the loss of membrane-bound digestive enzymes.[32,33] Influx of lymphocytes, eosinophils, and plasma cells occur in the lamina propria. These alterations reduce the capacity of the small intestine to absorb water, nutrients, and electrolytes.[33]

Cystoisospora belli is the etiological agent of cystoisosporiasis, a severe clinical condition among immunocompromised patients such as HIV, transplant, lymphomas, and leukemia patients, and has also been linked to traveler's diarrhea.[34] Although ecological data showed high rates of COVID-19 infection in high-income regions,[35] cystoisosporiasis was also linked to reduced quality of life.[36]

Interestingly, about half of COVID-19 patients experience GI symptoms, frequently preceding respiratory symptoms.[37] By binding to angiotensin-converting enzyme 2 (ACE-2) receptors, SARS-CoV-2 infects alveolar cells in the respiratory system. These receptors can be found on the surface of enterocytes,[38] where they play an essential role in microbiota homeostasis and mucosal inflammation.[39] According to virologists, most diarrhea-related viruses (i.e., rotavirus, adenovirus, and norovirus) are non-enveloped, making them more resistant and having a higher survival rate in the intestinal tract. The intestinal tropism of SARS-CoV-2, on the other hand, has been examined and found to have considerably higher resistance than other enveloped viruses in the intestinal tract.[40,41] SARS-CoV-2 RNA fragments have been found in stool samples, but not the entire virus.[42,43] SARS-CoV-2 was found to infect enterocyte lineage cells in a human intestinal organoid model.[44] Unfortunately, insufficient data are available to support this hypothesis in vivo due to the scarcity of autopsy investigations and practical constraints on GIT endoscopic examinations. SARS-CoV-2's ability to survive the acidic stomach environment and directly infect enterocytes is still unknown. Overall, it is uncertain

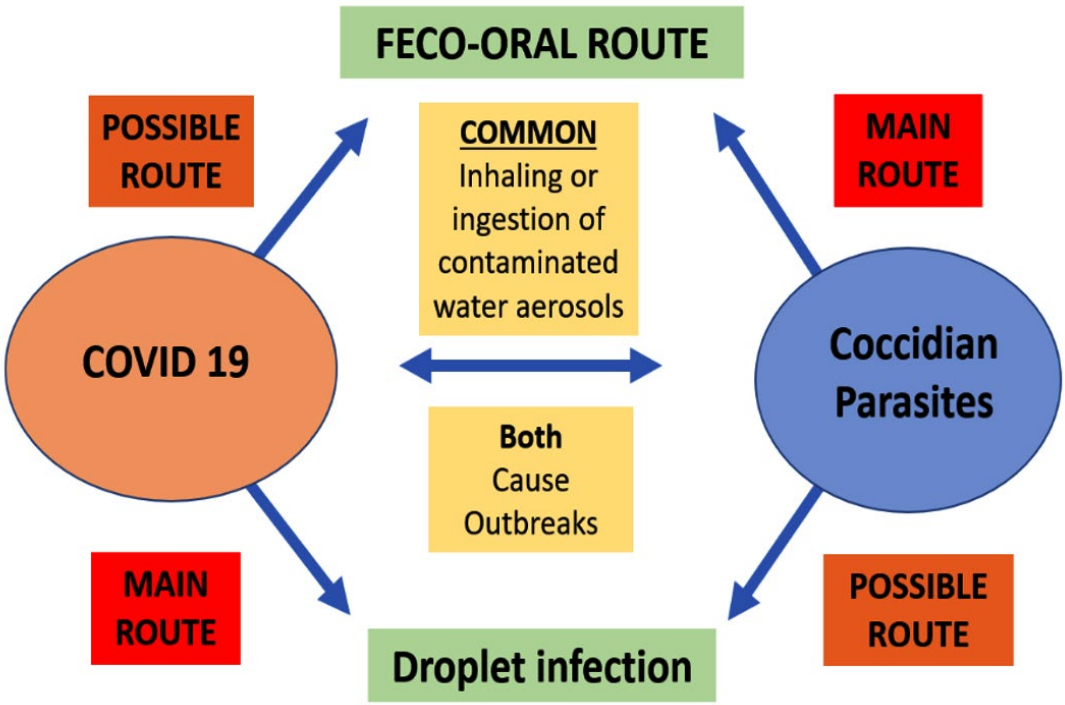

Figure 2. Shared routes of transmission between COVID-19 and Intestinal Coccidian parasites

if COVID-19' GI symptoms are caused by a primary GIT infection or other processes.[45] To the best of our knowledge there is no available data on whether ACE-2 receptors can play a role in the pathogenesis of intestinal coccidian's.

COVID-19 and Intestinal Coccidian Parasites: Do they Share a Way of Transmission?

The fecal-oral pathway, which involves ingesting oocysts from contaminated food and drink, is the most common way for coccidian parasites to spread. They are frequent in locations with inadequate sanitation, where diseased people's excrement has contaminated the environment.[46-48] COVID-19 is generally transmitted via two main routes; the principal pathway is respiratory droplets in the form of aerosols emitted by infected patients while sneezing or coughing, posing a risk of infection to others in close proximity.[49] Another pathway is contact transmission, in which respiratory droplets fall on surfaces and infect healthy people when they come into contact. This is in line with prior research on the transmission of several CoVs via contaminated aerosols and water droplets.[50] It was thought that *Cryptosporidium* could spread upon inhaling or ingestion of contaminated water aerosols in a way close to the transmission of various CoVs.[51]

In cryptosporidiosis, the transmission via respiratory secretions has also been postulated. Contact with fomites contaminated by coughing or inhalation of aerosolized droplets is assumed to be the routes of transmission for *Cryptosporidium* spp. oocysts.[52] Respiratory cryptosporidiosis has been discovered in up to one-third of children with diarrhea. Furthermore, respiratory disease increases the risk of primary respiratory infection with *Cryptosporidium* spp. in some patients who do not have intestinal involvement, leading to person-to-person transmission.[53]

Given the evidence of SARS-CoV and MERS-CoV causing wastewater contamination through human waste, the fecal-oral pathway can never be ruled out.[54,55] The existence of SARS-CoV-1 in wastewater identified in the Hong Kong (2003) outbreak due to improper sewage disposal.[54,56-58] has drawn attention to another potential source of infection. Consequently, attention was paid to this source in the COVID-19 pandemic, particularly after the first declaration made by Holshue et al. of detecting SARS-CoV-2 in human waste.[59] SARS-CoV-2 has been found in urban wastewater in a growing number of investigations either treated or untreated, to follow the virus's tracks and prevent the potential public health hazards linked to wastewater contamination with SARS-CoV-2.[60,61] The pathogen survival in wastewater systems depends on multiple variables such as temperature, pH, average in-sewer travel time, per-capita water use, average travel time, and the process of disinfection.[62-64] Up to 90% of pathogens are successfully removed via wastewater manipulation. However, additional treatments, for example, chlorination, are needed to reduce pathogens to levels that are safe to release into the environment.[65] SARS-CoV-2, an enveloped virus-like other coronaviruses,[50,66] is considered more delicate than the non-enveloped viruses and so easily damaged by solvent and detergents found in wastewater.[57] Previous studies have shown that SARS-CoV-1 is highly affected and completely inactivated by chlorination.[54] Furthermore, SARS-CoV-2 RNA in raw wastewater is rendered negative in tertiary-treated wastewater.[67,68] *Cryptosporidium* spp. is also among the globally distributed protozoa reported in wastewater. It makes a significant contribution to the burden of water-borne disease.[69] The existence and distribution of *Cryptosporidium* spp. oocyst in untreated wastewater are proportionally linked to the extent of infection.[70,71] Wastewater disinfection of *Cryptosporidium* spp. is markedly variable. It is frequently determined by the temperature and process of wastewater treatment. The most efficient disinfection methods include Ultrafiltration and UV disinfection combined with advanced oxidation.[72]

In a subset of patients, SARS-CoV-2 shedding has been recently detected in stool samples. However, the presence of live virus in fecal matter does not necessarily imply the existence of viral-RNA.[73] Scientists worldwide have studied this problem, and some of them found live SARS-CoV-2 in feces samples, confirming the possibility of transmission.[74] The use of ACE2 receptors by SARS-CoV-2 to enter the host's body is well known.[75,76] Multiple copies of viral RNA and proteins are then produced in the host cytoplasm, resulting in new virions that can spread throughout

the gastrointestinal system.[77] The gastric, rectal, and duodenal epithelia of COVID19 patients showed strong immune-fluorescent labeling of viral nucleocapsid protein and viral host receptor ACE2.[78] The probability of SARS-CoV-2 fecal-oral transmission in the current global outbreak is supported by these findings.[78,79]

Furthermore, live SARS-CoV-2 was found in blood samples and anal swabs, while oral swabs were negative for the same patients in a few cases. As a result, although posing a risk to others through fecal-oral transmission, those individuals were found to be COVID-19 negative during routine surveillance (Figure 2).[38] Also, saliva may contain SARS-CoV-2, viral spread can occur through aerosol transmission or water contaminated with infected saliva either if unknowingly used to wash hands/face by healthy individuals or a drinking water source is mixed with infected saliva.[80]

Outbreaks of cryptosporidiosis and cyclosporiasis have become evident in many different parts of the world owing to the enhancement in disease investigation's in recent years. Cryptosporidiosis outbreaks are linked to swimming pools or contaminated drinking water due to the high resistance of the oocyst to chemicals used in methods of water system disinfection such as chlorine, chloramines, and chlorine dioxide.[46-48,81] Simultaneously, cyclosporiasis outbreaks are typically caused by uncooked fresh vegetables that are contaminated by feces as they grow low to the ground. Outbreaks occur due to the consumption of raspberries, blueberries, snow peas, blackberries, basil, cilantro, strawberries, snap peas, and various lettuces.[46,47] However, the real cause of the outbreak is usually unknown; because the products are generally consumed or expired and discarded before the discovery of the outbreak.[47]

As a result, given the high SARS-Cov-2 load in COVID-19 patients' sputum, saliva, and stool samples, a substantial amount of wastewater released by quarantine centers, hospitals, residential households in areas with positive COVID-19 cases can easily be contaminated.[49] Vector born transmission can be questioned as well, COVID-19 is thought to be transmitted via cockroaches and houseflies, who might carry the virus if they came in contact with contaminated feces.[82] So far, a lack of sanitary standards in parasite-endemic areas of Low- and Middle-Income Countries (LMICs) has raised the danger of SARS-CoV-2 transmission and infection.[12]

Can COVID-19 Infection Mimic the Symptoms of Intestinal Coccidian Parasites?

Cryptosporidiosis is the second most common cause of diarrhea in children worldwide.[83] *Cryptosporidium* spp. infection is acute and self-limiting in patients infected with *C. hominis* and *C. parvum*, with symptoms appearing in approximately a week and might progress to extended or chronic diarrheal episodes more frequently than other enteric infections.[27] It might be severe in immunocompromised people.[84] Moreover, long-term development and cognitive problems can be caused by repeated infections.[85] The symptoms of human respiratory cryptosporidiosis are comparable to those of other common respiratory infections, despite its rarity. Inflammation of the sinuses, larynx, nasal mucosa, and trachea, as well as voice alterations and nasal discharge, are all symptoms of upper respiratory cryptosporidiosis.[52] A productive cough, dyspnea, fever, and hypoxemia, mark cryptosporidiosis of the lower respiratory tract.[52] Variations in susceptibility to and severity of *C. parvum* infections are observed but are not fully recognized. These multifactorial variations include the host's immunological status, previous exposure, genetics, and nutrition.[86]

The manifestation of cyclosporiasis varies according to the host's age and immune status and the infection's local endemicity.[33] Infection is typically mild or asymptomatic, especially among residents of highly endemic regions.[46] Symptoms include low-grade fever, fatigue, anorexia, nausea, profuse watery diarrhea, and abdominal pain, leading to weight loss. The stool may contain blood or mucus less frequently. The disease can take a severe form among infants, the elderly, and patients who are severely immunocompromised, such as those with HIV/AIDS.[33] Prolonged diarrhea can cause dehydration, malnutrition, and, in rare circumstances, severe dehydration and death in infants, despite being rarely lethal in humans.[87]

Travelers from countries where the disease is not endemic are also at risk of developing a severe infection. In immunocompromised people, *C. cayetanensis* can cause acalculous cholecystitis by infecting the biliary tract. Guillain-Barre syndrome, reactive arthritis, ocular inflammation, and sterile urethritis were other reported conditions.[33,88,89] Despite the absence of *C. cayetanensis* infection in the respiratory tract, oocysts were detected in two tuberculosis patients' nasal secretions.[87] In immunocompetent people, cystoisosporiasis is asymptomatic or causes self-limiting diarrhea.[90] On the other hand, it is presented with more severe, prolonged diarrhea and might have extra-intestinal manifestations such as liver, spleen, gallbladder, and biliary tract among immunosuppressed patients.[90,91]

SARS-CoV-2 targets the respiratory system, causing symptoms similar to the flu, such as fever, asthenia, and coughing.[2] Occasionally, these symptoms are associated with a loss of taste or smell. SARS-CoV-2 infection, on the other hand, has been related to a variety of digestive symptoms in children, such as vomiting, diarrhea, stomachache, and nausea.[92] While most adult patients present with respiratory and digestive symptoms, only a small percentage present solely with respiratory symptoms.[93] Infections with SARS-CoV-2 in the gastrointestinal tract can induce bleeding and inflammation, damaging the intestinal immune system before migrating to the rest of the body and exacerbating COVID-19 disease in the lungs and other organs.[94] In severe cases, it may be complicated by multi-organ failure, acute respiratory distress syndrome (ARDS), and sepsis. The disease severity is mainly seen in patients with risk factors such as the elderly, immunocompromised, and underlying comorbidities.[95-98] Pregnant women are more prone to develop severe troubles and die as a result of viral infections,[99] owing to physiological changes in the immune and respiratory systems, which are more prone to the severity of SARS-C0V-2 disease.[99,100] Intestinal parasites that compete with the host for nutrients might also affect a woman's nutritional condition and cause intestinal inflammation, reducing nutrient absorption.[101]

Does Parasite-driven Immune Regulation have a Beneficial or Detrimental Role in COVID-19 Infections?

It has been demonstrated that chronic parasitic infections could affect the clinical outcomes of other diseases, possibly as a result of direct manipulation of the host's immunological responses.[22,23] Therefore, pre-existing parasitic infections might either have a beneficial or detrimental effect on the host's immunological response to SARS-CoV-2 infection.[14,15,102] Recent research has shown that parasitic infection-induced immunity changes can influence the progression of viral diseases, in particular.[103] Chronic parasitic infections of the intestine are frequently linked to the development of alternatively activated macrophages (M2), type 2 innate lymphoid cells, and T helper-2 (TH2)-skewed in association with the induction of cytokines such as IL-4, IL-5, IL-13, and enhanced eosinophilia, and IgE responses.[22,23] The TH2 immune response is also associated with the production of a significant T cell regulatory (Treg) response, which is essential for the parasite's survival and chronic persistence and may influence responses to heterologous infection.[12,22,23] Parasite-driven TH-2 response is also essential for managing parasitic infections and repairing tissue damage triggered by the infections.[22,23]

Nevertheless, COVID-19 immune response is characterized by Th1 response with the activation of Macrophages, T-cytotoxic and the secretion of pro-inflammatory cytokines as TNF-α and IFN-γ.[104] Severe cases of COVID-19 were attributed to cytokine storms caused by over activation of pro-inflammatory cytokines such as detected IL-6.[9,14] Many scientific reviews described the potential role of pre-existing helminth infections on COVID-19. They suggested that helminthic infection can cause modulation of the immune response due to boosted Th2-like cytokine responses with the secretion of immunomodulatory cytokines (TGFβ and IL-10), and the induction of Treg cells.[15,102] It might decrease the uncontrolled Th1 proinflammatory cytokine response which is responsible for the severe manifestations of COVID-19. Therefore, persistent parasite-driven TH2 and Treg responses might be able to counterbalance the overactive TH1 responses described in severe COVID-19.[105]

According to Fonte et al.,[16] helminthic infections can also improve antiviral mechanisms resulting in better viral load control. This would be especially evident in countries where helminthic infections are prevalent, as there is a probability that helminthic infection could alter infection outcomes by altering the TH2 response to reduce the inflammatory component.[106] On the other hand, some researchers have attributed the delay in virus clearance due to activation of Th2 which secrete cytokines such as IL-4, IL-5, IL-13, and IL-10.[107] Hence, the contribution of type 2 response in the immunopathology of COVID-19 is of concern when considering the potential effects of helminth co-infection.[102]

Moreover, COVID-19 infection is known to cause exhaustion of the immune system, with progressive lymphopenia, depletion of macrophages, monocytes, and dendritic cells.[108] Likewise, systemic steroids used in treatment of some patients with severe COVID-19 could have an inhibitory effect on the inflammatory response. This might favor the co-infection with opportunistic parasitic infections including coccidian parasites which can be masked due to the overlapping with the GIT symptoms of COVID-19 infection.[25] The ability of parasitic infections in suppressing the outcome of the viral diseases was demonstrated in animal models giving the name of "parasites against virus phenomenon''. It was visible in some helminthic and protozoal infections.[109] Researches included the ability of *Trichinella spiralis* to lesser the immune-pathological changes caused by influenza A virus, the role of *Giardia lamblia* in reducing the severity of rota viruses' infection and the protective effect hypothesized between *Plasmodium* spp. and Chikungunya virus.[110-112] Other protozoal diseases that were highlighted in COVID-19 is the co-infection in Malaria endemic areas. As with COVID-19, malaria involve cellular immune responses with the release of pro-inflammatory cytokines including interferon-gamma, TNF-alpha, IL-6, and IL-12.[14] This occurs in a way that may suggest an excessive pro-inflammatory response in case of co-infection with COVID-19. On the other hand, studies in malaria-endemic regions have found that Malaria can induce immunosuppression, inhibiting the immune response to other infections. This was highlighted in co-infection with *Salmonella* spp.[113,114] and the given protection against severe manifestations of some respiratory viruses.[115] All of which may indicate a similar role in decreasing the severity of manifestations in COVID-19. Moreover, other studies indicated that *Plasmodium* spp. and SARS-CoV2 use a common CD147 receptor to enter the cell and have resemblances in their MHC-presented antigenic determining factor, which also suggest that immunity against parasitic infections can give protection against SARS-CoV-2 infections.[103]

Questioning the interaction between COVID-19 and Coccidian parasites, in particular, which are known for being opportunistic. Relevance of innate immunity in cryptosporidiosis has been underlined in several recent research on intestinal coccidiosis. In cryptosporidiosis, intestinal epithelial cells are crucial because they are the only host cells for parasite reproduction and contribute to the protective immune response. Immune cells are drawn to infected areas by chemokines produced by epithelial cells. They also secrete parasiticidal antimicrobial peptides that cause apoptosis. Intestinal dendritic cells enhance adaptive immunity and control *C. parvum* in the early phases of infection.[116] In order to control cryptosporidiosis, both innate and adaptive immune responses are required. Innate immune responses are influenced by TLR pathways, antimicrobial peptides, prostaglandins, cytokines, mannose-binding lectin, and chemokines. It is impossible to overestimate the impact of cell-mediated responses, particularly those involving CD4+ T cells and IFN- γ. According to Tessema et al.[117] immunity development in *Cryptosporidium* spp. infected humans and mice was associated with increased expression of IFN-γ T1 cytokines, and in order to slow down these protective measures, the parasite has created a range of escape mechanisms.[118]

The host mounts an innate and adaptive immune response to *C. cayetanensis* invasion when sporozoites are liberated from the oocyst in the small intestine. The sporozoite infects the enterocytes in that location, releasing cytokines and activating and recruiting phagocytes from the bloodstream to the infection site. Several factors are secreted by these phagocytes, which increase intestinal chloride and water secretion while inhibiting absorption.[119] The parasite and

inflammation can cause direct damage to the enterocytes. T cells, protease, and oxidants secreted by mast cells are blamed for this. Watery diarrhea is triggered by enterocyte destruction, nutrient malabsorption, and increased fluid and electrolyte secretion.[119]

Therefore, the Th1 response seen in *Cryptosporidium* spp. infection, with the production of TNF-α, IL-2 and IFN-γ may question its role in exacerbating the manifestation of COVID-19 in case of their co-infection.[120] On the other hand, in experiments using *Cryptosporidium* spp. and Bovine Coronavirus, *Cryptosporidium* infection increased the entry of the virus inside cells by its attachment to the parasite during coincubation, but the virus replication was inhibited. One of the explained theories was the presence of the parasitophorous vacuole of the parasite which may surround the virus inhibiting its replication.[121] While the opposite was seen with HIV virus as replication of the virus was increased in *Cryptosporidium* spp. infection. Explaining another mechanism of action which is the increased replication of HIV- virus in activated CD4 generated by *Cryptosporidium* spp. infection.[122] While Abdel-Hamed et al.,[26] declared that *Cryptosporidium* spp. was more prevalent in COVID-19 patient with mild symptoms than in patients with severe symptoms, indicating a protective role of *Cryptosporidium* spp. They advocate this protection due to the immunological potential of the apicoplast proteins of the protozoa. More studies are needed to find correlation between Coccidian parasites and COVID-19 infection.

How can the Parasite-driven Microbiome Alteration Impact the Pathogenesis of COVID-19?

The human microbiome is essential for the formation and maintenance of immunological homeostasis, and it is well known that dysbiosis or imbalance of microbiota is strongly linked to a broad range of diseases. The oral cavity and the intestinal tract attain essential roles in the pathogenesis of infectious diseases as they contain the largest and second-largest microbiotas in the human body.[123] Individuals in HICs have a less diverse gut microbiome compared to those in LMICs, due to multiple variables, including improved sanitation, genetic differences, urban versus rural living, diet diversity, and antibiotic overuse.[21,22,124,125] So far, despite the limited number of studies, gut dysbiosis has been consistently displayed in COVID-19 patients. Determining how the gut microbiota influences different levels of COVID-19 severity is a more relevant clinical implication. As previously indicated, COVID-19's clinical spectrum extends from asymptomatic to severe, life-threatening illness.[126] Owing to the virus's novel nature, variations in clinical severity are less likely to be caused by the presence of less virulent strains or adaptive immune protection. The host's immune response is still the most likely determinant of disease severity. When infected with SARS-CoV-2, it is unknown whether any specific pattern of gut microbiota protects people from acquiring a severe inflammatory state.[45] Nevertheless, the elder people with chronic inflammation are more prone to develop a pro-dysbiotic state.[127,128] COVID-19 caused the highest rates of morbidity and mortality in the elderly, immunocompromised cancer patients, and those with underlying chronic medical illnesses, which is unlikely to be a coincidence.[45]

A self-perpetuating inflammatory feedback loop can occur when an additional inflammatory stimulus shifts the balance toward a leaky gut in people with gut dysbiosis. To their surprise, two studies discovered a relationship between gut dysbiosis and severe COVID-19.[129,130] Infection with SARS-CoV-2 may also increase susceptibility to bacterial infections, increasing illness severity and mortality.[123] When compared to healthy controls, the fecal microbiomes of COVID-19 patients had a proliferation of opportunistic pathogens and a loss of beneficial commensals, according to the shotgun metagenomics approach.[123]

According to most studies, the composition and diversity of microbiota secondary to protozoal or helminthic infections may benefit health in LMICs. At the same time, the decline in the rate of parasitic infections in HICs has resulted in a rise in the prevalence of chronic inflammatory conditions.[24] Several studies have found that intestinal parasites can influence the immunological response of their hosts by altering the gut microbiome's quantitative and qualitative composition.[24] Helminthic infection might also modulate the severity of viral infections through alteration in the gut microbiome composition

which can change immune response to viral infection.[24] Enteric helminths have previously been proven in animal models to prevent pulmonary viral infections by interacting with the microbiota.[131] Colonization of the intestine by increased variety and composition of beneficial gut microbiota has also been associated with *Blastocystis* spp. and *Entamoeba* spp. excluding the pathogenic *Entamoeba histolytica*.[132-134] Anti-inflammatory responses have been linked to parasite-induced microbiome alterations.[135]

In contrast, microbiota has become dysbiotic as a result of infection with giardiasis, which activated TLR signaling pathways and triggered an overproduction of proinflammatory cytokines such as IL-1α and IL-1β.[136] Alterations in gut flora have been demonstrated in both mice[137] and monkeys[138] following infection with *C. parvum*. Changes in gut flora, on the other hand, may impact the growth and infectivity of intestinal parasites. The patterns of microbiota in volunteers infected with *C. parvum* were studied and related to human vulnerability to cryptosporidial infection.[139] Antibiotic usage is a common cause of gut microbiome alteration. Despite the inability to identify the causative microbial agent, antibiotic therapy may be administered, which may not be effective for some protozoa that cause diarrhea, such as *Cryptosporidium* spp.[140] Actually, inappropriate use might worsen the condition in some cases. Accordingly, it is compelling to investigate the intestinal microbiome in conjunction with SARS-CoV-2 and intestinal coccidian co-infection, as well as the crosstalk between them and the immune system, as this might provide a better understanding of COVID-19 disease progression (Figure 3). Additionally, in our way to develop alternative treatments, experiments should also be designed to determine if probiotics can influence the course of co-infections. Understanding the immunology and consequences of SARS-CoV-2 infection in patients coinfected with intestinal coccidiosis especially in LMICs needs further research.

How can COVID-19 Pandemic Impact Parasitological Diagnostics and Eliminations Strategies?

Laboratory diagnosis and testing of relevant specimens obtained from patients who meet the COVID-19 suspected case definition are considered as a priority for adequate clinical management and containment of the COVID-19 pandemic.[141] To keep laboratory staff safe while delivering reliable test findings, proper precautions must be implemented. Antibody-based techniques have been presented as additional tools for determining the etiology of SARS-CoV-2 infection, with real-time reverse transcription-PCR (RT-

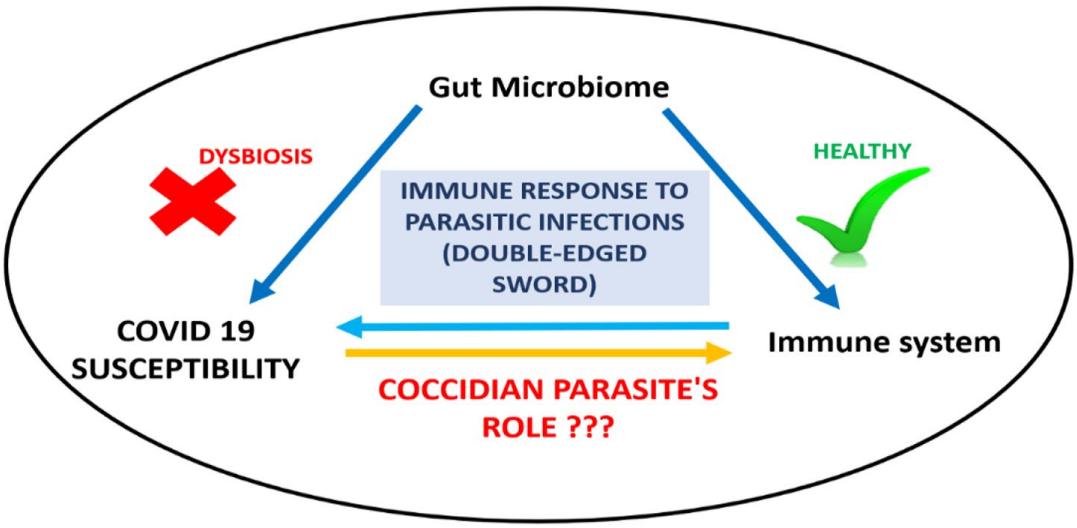

Figure 3. The crosstalk between the intestinal microbiome in conjunction with SARS-CoV-2, co-infection with intestinal coccidian, and immune system.

PCR) assays remaining the molecular test of choice.[142] According to Guchowska et al.,[1] the COVID-19 pandemic had a considerable effect on the diagnosed human parasitosis cases, as evidenced by a decrease in the ordered parasitological diagnostic tests and completed in numerous laboratories. Many techniques for detecting coccidian parasites have been developed, including molecular, immunological, and microscopic techniques. Immunological and molecular techniques require a longer time, are more complex, and are much more expensive, making them less useful for screening, especially in resource-constrained settings.[143] Microscopy can also be utilized for screening in primary care settings because it is a rapid, low-cost, and reliable diagnostic method. It has the advantage of giving direct visual confirmation of chemically and environmentally resistant oocysts in stool samples.[143] Notably, both clinicians and microbiologists should be aware of coccidian parasites among immunosuppressed patients complaining of prolonged diarrhea, and further investigations, such as modified acid-fast staining method and fluorescence microscopy, should be performed, as these parasites could be missed during routine coprological examination.[144]

Currently, there is no vaccination for cryptosporidiosis, and the Food and Drug Administration has only licensed one drug for immunocompetent individuals in the United States.[85] Similarly, no cyclosporiasis vaccine is available at this time. Preventative efforts, on the other hand, concentrate on increasing sanitation and food treatment to kill resistant oocysts. Travelers to highly endemic regions should avoid eating uncooked fresh vegetables and unpeeled fruits, preferring instead to eat completely prepared dishes.[145] Moreover, untreated water should be avoided by recipients of solid organ transplants and patients with HIV.[146] Hand washing after toilet use and before meals and proper disposal and treatment of human excreta are crucial for cyclosporiasis prevention.[87] From our understanding as parasitologists, we suggest that in patients diagnosed with COVID-19 and complaining of gastrointestinal manifestations, mainly diarrhea, stool analysis should be routinely required, and investigation for enteric protozoa, intestinal coccidiosis, in particular, ought to be done. This simple practice would give us an idea about the actual occurrence of this type of co-infections (parasitic and viral) in both developing and developed countries within the same patient/population.

Exploratory studies toward new protective perspectives and COVID-19 therapeutic techniques are vital, while proven efficiency of COVID-19 therapy and global mass vaccination are unanswered.[123] Similarly, the risk of SARS-CoV-2 infection are likely to be minimized by frequent hand washing and disinfection measures applied for the reduction of parasitic infections transmitted through dirty hands.[1] This was demonstrated in a study done comparing the prevalence of intestinal parasitic infections before and after COVID-19 pandemic demonstrating a decrease prevalence by 40% after COVID-19 pandemic. Indicating that improving sanitation and personal hygiene can be effective against parasitic infections in COVID-19 era.[147] Also, Knox et al.,[148] demonstrated that the lockdown period during COVID-19 pandemic interrupted the human-to-human transmission of *Cryptosporidium* hominis while the zoonotic transmission of *C. parvum* remain possible and they highlighted the possibility of controlling or even eradicating C. hominis through non-pharmaceutical interventions. But it is of concern that, due to convergence of all attention toward COVID-19, there is a pause in fighting parasitic infections which may cause increase incidences of malaria, leishmaniasis, schistosomiasis, and soil-transmitted helminths.[103]

From our point of view, SARS-CoV-2 vaccines have demonstrated a reduction in the severity of disease and improved survival of affected patients. Their effect has been observed in developed countries, where mass vaccination has been most efficient, while vaccination campaigns have been relatively slow in developing regions. Vaccine efficacy in underdeveloped nations, particularly those with high rates of chronic parasite diseases, remains a source of concern.

CONCLUSION AND FUTURE PERSPECTIVES

The SARS-CoV-2 virus, recently discovered, has been recognized as the cause of the COVID-19 pandemic. Although SARS-CoV-2 infection, like other coronavirus infections, is closely linked to

respiratory symptoms, COVID-19 patient datasets have revealed gastrointestinal symptoms such as diarrhea, vomiting, nausea, and abdominal pain. SARS-CoV-2 pathogenesis is better understood, and severe disease outcomes depend on the virus's ability to penetrate epithelial cells by binding to the ACE 2 receptor. This can cause a severe hyperimmune reaction in the host, as well as a potentially fatal cytokine storm in the lungs, resulting in systemic inflammatory response syndrome. Furthermore, the COVID-19 pandemic represents a challenge for public health systems worldwide, since it might impact other significant pathogens, including parasitic diseases, intestinal coccidian parasites, in particular. The present review highlighted the possible mutual influence between the intestinal coccidian parasites and COVID-19 disease. The available data suggest that coccidian parasites in the intestine can enhance the chance of COVID-19 transmission, subsequently its prevalence. In the current situation, more research is required to detect the frequency of virulent viral strains and fecal viral load, which are of concern crucial source for transmissibility.

Likewise, there is a possibility that the lack of parasite co-infection in developed countries might increase the severity of COVID-19, as parasitic infections can decrease COVID-19 severity through direct modulation of the immune system along with indirect parasite-driven microbiome balance. However, parasitic infections can damage the immune system, degrade tissue, promote malnutrition and microbiome dysbiosis, support virus infection, and reduce vaccination effectiveness. As a result, this study aimed to collect and integrate numerous data and demonstrate an overview of knowledge gaps that need to be addressed, besides suggesting some theories regarding the pathogenesis of COVID-19 infection, which continues to have a significant impact on population health. We questioned whether the presence of pre-existing coccidian parasitic infection may augment COVID-19 manifestations due to common release of proinflammatory cytokines, or whether it may cause inhibition of virus replication as occurred in other viruses. At the same time, a reciprocal effect of COVID- 19 was highlighted. The infection with COVID-19 may increase the occurrence of coccidian parasites due to depletion of the immune system favoring the increase of opportunistic infection, which is difficult to diagnose due to overlapping of symptoms. But the control measures, health education, hand hygiene and lockdown directed toward COVID-19 disease may decrease the transmission of coccidian parasites. All of which need further research. These findings might have far-reaching implications for disease management and infection prevention.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

FZ conceptualized the research idea. HMA and FZ designed the figures. HMA performed the supervision. HMA, MA and FZ wrote the manuscript. HMA reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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