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Review Article

Can Free Living *Acanthamoeba* Act as a Trojan Horse for SARS-Cov-2 on Viral Survival and Transmission in the Environment? A Narrative Review

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Abstract

Acanthamoeba is a free-living amoeba that has been found on surfaces, air, water and various environmental sources around the world. It enters the human body through the respiratory tract via the nose. Of note, amoebae are well known to act as a reservoir for various pathogenic microorganisms including bacteria and viruses such as *Adenoviruses* and *Mimivirus*. Given that SARS-CoV-2 of the Coronaviridae family is transmitted through the respiratory tract, and the Trojan horse nature of *Acanthamoeba*, it has been suggested that amoebae act as a vector in the transmission of SARS-CoV-2. The aim of this study was to provide an opinion of the possibility of the coexistence of *Acanthamoeba* with SARS-CoV-2.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first seen in Wuhan, China in December 2019 (1). The virus belongs to the *Coro-*

naviridae family, which has a double-stranded RNA genome (2, 3). Symptoms range from asymptomatic forms to mild colds and sometimes severe lung involvement, heart and kid-



ney involvement, central nervous system (CNS), and death (4, 5). The virus has shown exceptional transmission around the world (4). It reproduces intracellularly. One of the most important ways for viruses to survive is to escape the immune system through intracellular proliferation (6).

Free living amoeba (FLA) includes *Acanthamoeba*, *Naegleria fowleri*, *Balamuthia mandrillaris* and *Sappinia* (7). *Acanthamoeba* is an opportunistic protozoan that is widely found in soil, air, drinking water, pool water, surfaces, contact lenses, and hospital rooms, and almost everywhere (8, 9). This free-living protozoan has two forms: cyst and trophozoite (10). On average, humans are exposed to the cyst in their routine lives. *Acanthamoeba* cysts are very resistant to adverse environmental conditions due to the presence of cellulose in the inner layer of the form, including high temperature, variable pH and lack of nutrients (9). This protozoan does not need a host given its free-living nature. However, it has the ability to attack and feed on mammalian and human cells (11). Being airborne, the cyst form provides a route of transmission to humans (12). *Acanthamoeba* travels mainly through the lower respiratory tract and nose to the lungs and then to the brain parenchyma, and can be opportunistically pathogenic if the host is susceptible, i.e., weakened immune system (13). As a result, an attack on the body's cells can lead to the brain damage, skin lesions, keratitis, sinusitis and diffuse form of acanthamoebiasis (14).

Thus far, fungi, bacteria and viruses have been isolated from this amoebae as endosymbionts (7, 15). In fact, one of the most important free-living amoebae capable of harboring and phagocytic microorganisms is *Acanthamoeba* and it is also used as a research model in the laboratory (16). The trophozoite form has the ability to phagocyte a variety of microorganisms, including bacteria and viruses, and in some cases these microorganisms can remain viable in the cyst stage during encysta-

tion process, and thus cause survival of the microorganism within itself (17, 18). In this regard, *Acanthamoeba* is considered as the environmental phagocyte as it exhibits properties and associated molecular mechanisms that are remarkably similar to phagocytes. In support, *Acanthamoeba* has been shown to contain a variety of viral agents such as *Mimivirus*, *Adenovirus*, *Yaravirus*, *Coxsackievirus*, and a variety of bacteria such as *Pseudomonas*, *Legionella*, and *Mycobacteria* to name a few (8, 19-26).

One of the most important ways of transmitting *Acanthamoeba* to humans is through the respiratory tract via the nostrils, and its clinical course varies from asymptomatic to fatal encephalitis (6). Likewise, SARS-CoV-2 enters the human body in the same way (27). These findings suggest that *Acanthamoeba* may act as a potential host for SARS-CoV-2 to shelter, multiply and survive, and allow viral transmission to the susceptible hosts. The ubiquitous nature of *Acanthamoeba*, together with the hardy nature of its cyst form, further support this speculation, i.e., SARS-CoV-2 could use *Acanthamoeba* as a transmitter and reservoir (1, 28). Therefore, it is hypothesized that the virus may reside inside amoebae as a hyper-parasite.

SARS-CoV-2 was first reported in Iran in 2020 in Qom Province and is one of the countries that has been affected heavily by the SARS-CoV-2 pandemic and entered the fifth peak (29, 30).

The total number of known patients are 4556417 so far and 99691 are the total number of veterans until August 18, 2021 in Iran (31). Iran's first peak was reported on March 1, 2020, and was recognized worldwide as a pandemic in early March (WHO) (32). Lack of social distance, not wearing a mask, frequent trips to epidemic centers can be the most important reasons for an increase in statistics and the number of peaks (33). One of the most important reasons for the spread of this virus is asymptomatic carriers and also the relatively low sensitivity of tests to distinguish healthy people from infected people. All SARS-CoV-2

variants, including alpha, beta, gamma, delta and omicron strains, have been detected in Iran (34).

Acanthamoeba in Iran has been frequently isolated from environments such as air, hospital surfaces, water and soil (17, 35-37). Therefore, a comprehensive study of the presence of these two pathogens together in one place and the carrier nature of *Acanthamoeba* is perhaps important for SARS-CoV-2 and worth investigating with an eye to design preventa-

tive strategies. Therefore, the purpose of this opinion study is the possibility of *Acanthamoeba* hosting of SARS-CoV-2.

Fig. 1 describes how *Acanthamoeba* and SARS-CoV-2 entered to host and interact together. Which may lead to the *Acanthamoeba* acting as host or Trojan horse. On the left-hand side, *Acanthamoeba* intake of SARS-CoV-2. On the right-hand *Acanthamoeba* with SARS-CoV-2 transmitted to host by respiratory system.

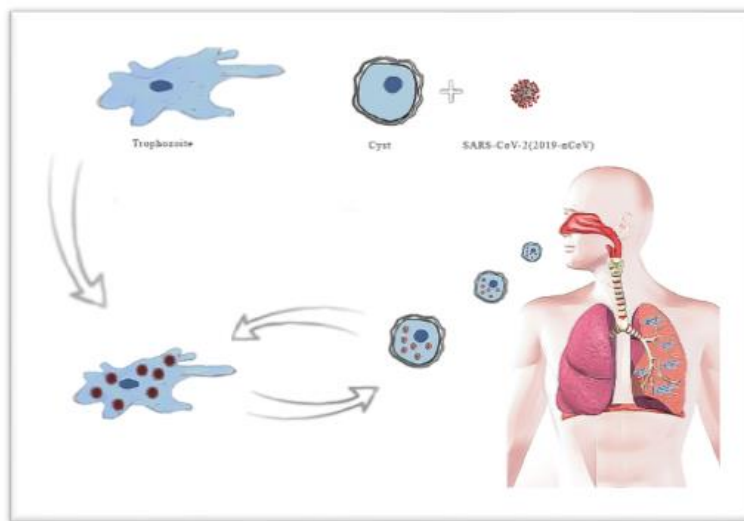


Fig. 1: Possible interaction of *Acanthamoeba* with SARS-CoV-2

Discussion

Acanthamoeba in nature can be a good repository and host for a wide range of bacteria and viruses (7). The most important bacteria reported with *Acanthamoeba* are *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Caedibacter caryophyllus*, *Francisella tularensis*, *Chromatium vinosum*, *Escherichia coli* and *Klebsiella* (7, 20, 38-41). These bacteria grow and multiply well in *Acanthamoeba*. Studies have shown that *Acanthamoeba* can cause drug resistance in some bacteria (15, 41). Bacteria and viruses can hide inside *Acanthamoeba* and escape digestion by immune cells. *Acanthamoeba* has a very strong ability to protect its endosymbionts, which allows them to grow and survive (41). This is especially

true of viruses for hosting, and the impact on their pathogenicity can be significant.

Acanthamoeba as viral vector

In recent years, many cases of *Acanthamoeba* have been reported to carry viruses, including *mimivirus*, *Adenovirus*, *Rotavirus*, *Marseillevirus*, *Mamavirus*, *Coxsackie virus*, and *Poliovirus* (42-44). Boratto et al. reported *Acanthamoeba* as a carrier of *Yaravirus* (25). The virus is 80 nanometers (about the size of a SARS-CoV-2) that contains dsRNA and also contains hundreds to thousands of genes encoding it. Most viruses isolated from free-living amoebae belong to the Nucleocytoplasmic Large DNA Viruses (NCLDV), which belong to the family of eukaryotic viruses that include the families *Phy-*

codnaviridae, Iridoviridae, and Asfarvirid (14, 45, 46). Most viruses reported with *Acanthamoeba* are large, encoding from 100 to 1,000 genes. Another study reported the coexistence of *Mimivirus* with *Acanthamoeba* (47). Previously, the two organisms were first seen together in 2003 under the name *Acanthamoeba polyphaga Mimivirus* (48). The *Mimivirus* consists of a nucleus containing the dsDNA genome, which is surrounded by a lipid membrane with a capsid size of 120 nm (49). The extent of increased pathogenicity in *Acanthamoeba* containing the virus and the virus itself is not well understood and needs further investigation.

Environmental Proximity of SARS-CoV-2 and *Acanthamoeba*

SARS-CoV-2, which causes COVID-19, is a new virus in the family of *Coronaviridae* that causes acute respiratory syndrome (50). The virus has a dsRNA genome, which has a high genetic diversity. The most important routes of transmission of SARS-CoV-2 include coughing, sneezing, close contact, kissing, and to a lesser extent through contact with infected surfaces (51). It is about 50 to 200 nanometers in size and is currently one of the most important viral infections in humans due to its relatively high prevalence and associated mortality (52). Due to the presence of SARS-CoV-2 in the environment, air and surfaces and the simultaneous presence of *Acanthamoeba* in these places, it is therefore possible for *Acanthamoeba* to harbor the SARS-CoV-2 and this area of research is ought to be investigated.

In a study, a similar role of macrophages and *Acanthamoeba* was noticed. Due to the high similarity in function and behavior of *Acanthamoeba* and macrophages, this amoeba could be a potential host for SARS-CoV-2 (6). In another study, SARS-CoV-2 could be delivered to the brain by *Acanthamoeba*. Due to the ability of *Acanthamoeba* to attack the brain parenchyma and the occurrence of encephalitis, SARS-CoV-2 can also enter *Acanthamoeba* in the environment and attack the brain with it.

Therefore, this issue is very important, because this virus can easily spread to different parts of the body with *Acanthamoeba* and cause serious complications (27).

Also, *Acanthamoeba* is a potential repository for carrying SARS-CoV-2. Given the explanations for the same release and entry of these two microorganisms, this is not unexpected (1).

Why SARS-CoV-2 is difficult to eradicate?

One of the important issue is SARS-CoV-2 is difficult to eradicate, possibly due to hiding inside *Acanthamoeba* which is resistant to disinfectants. This possible relation of SARS-CoV-2 with *Acanthamoeba* may provide protection of virus from external interventions and a dispersal mechanism across various habitats (53). It is still unclear that SARS-CoV-2 is an intracellularly-replicating or -residing organisms. As mentioned earlier, *Mimiviruses*, *Pandoraviruses*, and *Pithoviruses* are some examples for viral endocytobionts within FLA. Future research including co-cultivation studies, genomic, transcriptomic, and proteomic analyses are needed to clarify this issue.

In studies of *Acanthamoeba* viruses, some studies have reported that isolated viruses have so far detected at the cyst and trophozoites form (26, 54). Therefore, more research is needed on how *Acanthamoeba* function and interact with SARS-CoV-2, and no study has been conducted in this regard, although in the case of bacteria, the hypothesis that *Acanthamoeba* is capable of swallowing and phagocytosis has been fully established. It also increases the pathogenicity and even drug resistance in bacteria (55).

With the possibility of endosymbiosis of SARS-CoV-2 in *Acanthamoeba* and the possibility of survival up to 20 years, coupled with the relatively high resistance of SARS-CoV-2 to environmental conditions, it is feasible to consider that *Acanthamoeba* cysts could transmit SARS-Cov-2 into the human body and cause infection.

In the host body, trophozoites together with SARS-CoV-2 can lead to the defeat of macrophages and the virus can easily multiply in the host body. In a study of CVB3 with *Acanthamoeba*, virus-associated trophozoites killed macrophages due to the secretion of infectious material from the virus (26). Therefore, much research is needed to document this in relation to SARS-CoV-2 and *Acanthamoeba*.

Conclusion

Acanthamoeba have been found to phagocytosis fungi, bacteria, and viruses and serve as a repository for these endosymbionts. This brief review of the literature found five peer-reviewed studies discussing the interactions of *Acanthamoeba* and SARS-CoV-2. Although no studies have documented evidence of SARS-CoV-2 as an endosymbiont, but the literature suggests it could be possible due to the size of the virus, similar modes of infection, ubiquitous environmental presence of both organisms, and documented infections of hosts via *Acanthamoeba* with other viruses. There is a significant amount of research still needed to understand the interactions between *Acanthamoeba* and SARS-CoV-2.

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Conflict of interest

The authors declare that they have no conflict of interest.

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