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.

Keywords: SARS-CoV-2; *Acanthamoeba*; Interaction

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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 *Coronaviridae* 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 encystation 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*, *Coxackievirus*, 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 preventative 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 caryophi-lus, 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 Marseillele-virus, Mamavirus, Coxsackie virus, and Poliivirus (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 Nucleocyttoplasmic Large DNA Viruses (NCLDV), which belong to the family of eukaryotic viruses that include the families _Phy-
**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 issues 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.

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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 SASR-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.

**References**

1. Matin A. *Acanthamoeba*: A Potential Reservoir for Environmental Transmission of COVID-19 with Public Health Significance: Pros and Cons. Open Microbiol J. 2020;14(1):130-31.
2. Sardar R, Satish D, Birla S, Gupta D. Comparative analyses of SAR-CoV2 genomes from different geographical locations and other coronavirus family genomes reveals unique features potentially consequential to host-virus interaction and pathogenesis. Heliyon. 2020;6(9):e04658.
3. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265-9.
4. Li H, Xue Q, Xu X. Involvement of the nervous system in SARS-CoV-2 infection. Neurotox Res. 2020;38:1-7.
5. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
6. Siddiqui R, Khan NA. Can the Environmental Phagocyte *Acanthamoeba* Be a Useful Model to Study SARS-CoV-2 Pathogenicity, Infectivity, and Evasion of Cellular Immune Defenses? ACS Chem Neurosci. 2020;11(15):2149-51.
7. Mungroo MR, Siddiqui R, Khan NA. War of the microbial world: *Acanthamoeba* spp. interactions with microorganisms. Folia Microbiol (Praha). 2021;66(5):689-699.
8. Scheid P, Schwarzenberger R. *Acanthamoeba* spp. as vehicle and reservoir of adenoviruses. Parasitol Res. 2012;111(1):479-85.
9. Khan NA, Siddiqui R. Predator vs aliens: bacteria interactions with *Acanthamoeba*. Parasitology. 2014;141(7):869-74.
10. Gonçalves DdS, Ferreira MdS, Gomes KX, Rodríguez-de La Noval C, Liedke SC, da Costa GCV, et al. Unravelling the interactions of the environmental host *Acanthamoeba castellanii* with fungus through the recognition by mannose-binding proteins. Cell Microbiol. 2019;21(10):e13066.
11. Sriram R, Shoff M, Booton G, Fuerst P, Visvesvara GS. Survival of *Acanthamoeba* cysts after desiccation for more than 20 years. J Clin Microbiol. 2008;46(12):4045-8.

12. Fanselow N, Sirajuddin N, Yin X-T, Huang AJ, Stuart PM. *Acanthamoeba* keratitis, pathology, diagnosis and treatment. Pathogens. 2021;10(3):323.

13. Abrahão JS, Dornas FP, Silva LC, et al. *Acanthamoeba* polyphaga mimivirus and other giant viruses: an open field to outstanding discoveries. Virol J. 2014;11(1):1-12.

14. Reteno DG, Benamar S, Khalil JB, et al. Faustovirus, an asfarvirus-related new lineage of giant viruses infecting amoebae. J Virol. 2015;89(13):6585-94.

15. Greub G, Raoult D. Microorganisms resistant to free-living amoebae. Clin Microbiol Rev. 2004;17(2):413-33.

16. McCuddin Z, Carlson S, Rasmussen M, Franklin S. *Klebsiella to Salmonella* gene transfer within rumen protozoa: implications for antibiotic resistance and rumen deaunaft. Vet Microbiol. 2006;114(3-4):275-84.

17. Pazoki H, Niyyati M, Javannard E, Spotin A, Mirjalali H, Behravan MR. Isolation and phylogenetic analysis of free-living amoebae (*Acanthamoeba, Naegleria, and Vermamoeba*) in the farmland soils and recreational places in Iran. Acta Parasitol. 2020;65(1):36-43.

18. Siddiqi R, Khan NA. War on terror cells: strategies to eradicate “novel coronavirus” effectively. ACS Chem Neurosci. 2020;11(9):1198-9.

19. Holden EP, Winkler H, Wood D, Leinbach E. Intracellular growth of *Legionella pneumophila* within *Acanthamoeba castellanii*. Infect Immun. 1984;45(1):18-24.

20. Dey R, Rieger AM, Stephens C, Ashbolt NJ. Interactions of *Pseudomonas aeruginosa* with *Acanthamoeba* polyphaga observed by imaging flow cytometry. Cytometry A. 2019;95(5):555-64.

21. Adékambi T, Ben Salah S, Khalif M, Raoult D, Drancourt M. Survival of environmental mycobacteria in *Acanthamoeba* polyphaga. Appl Environ Microbiol. 2006;72(9):5974-81.

22. Suhre K. Gene and genome duplication in *Acanthamoeba* polyphaga Mimivirus. J Virol. 2005;79(22):14095-101.

23. Bekliz M, Azza S, Seligmann H, Decloqueumen P, Raoult D, La Scola B. Experimental analysis of mimivirus translation initiation factor 4a reveals its importance in viral protein translation during infection of *Acanthamoeba polyphaga*. J Virol. 2018;92(10):e00337-18.

24. Lorenzo-Morales J, Coronado-Alvarez N, Martínez-Carretero E, Maciver SK, Valladares B. Detection of four adenovirus serotypes within water-isolated strains of *Acanthamoeba* in the Canary Islands, Spain. Am J Trop Med Hyg. 2007;77(4):753.

25. Boratto PV, Oliveira GP, Machado TB, et al. Yaravirus: A novel 80-nm virus infecting *Acanthamoeba castellanii*. Proc Natl Acad Sci U S A. 2020;117(28):16579-86.

26. Mattana A, Serra C, Mariotti E, Delogu G, Fiori PL, Cappuccinelli P. *Acanthamoeba castellanii* promotion of in vitro survival and transmission of coxsackie b3 viruses. Eukaryot Cell. 2006;5(4):665-71.

27. Baig AM. Can neurotropic free-living amoeba serve as a model to study SARS-CoV-2 pathogenesis? ACS Chem Neurosci. 2020;11(22):3697-700.

28. Muhammad JS, Siddiqui R, Khan NA. COVID-19: Does SARS-CoV-2 Modulate *Acanthamoeba* Epigenetics to Enhance Survival and Transmission in the Environment? ACS Pharmacol Transl Sci. 2021;4(2):1021-3.

29. Hashemi SA, Safamanesh S, Ghasemzadeh-moghaddam H, Ghaifouri M, Azimian A. High prevalence of SARS-CoV-2 and influenza A virus (H1N1) coinfection in dead patients in Northeastern Iran. J Med Virol. 2021;93(2):1008-12.

30. Arab-Mazar Z, Sah R, Rabaan AA, Dhama K, Rodriguez-Morales AJ. Mapping the incidence of the COVID-19 hotspot in Iran—Implications for Travellers. Travel Med Infect Dis. 2020;34:101630.

31. Pourmalek F, Hemami MR, Janani L, Moradi-Lakeh M. Rapid review of COVID-19 epidemic estimation studies for Iran. BMC Public Health. 2021;21(1):1-30.

32. Organization WH. WHO announces COVID-19 outbreak a pandemic. 2020.

33. Khademian F, Delavari S, Koochiani Z, Khademian Z. An investigation of depression, anxiety, and stress and its relating factors.
during COVID-19 pandemic in Iran. BMC Public Health. 2021;21(1):1-7.
34. Taghizadeh P, Salehi S, Heshmati A, et al. Study on SARS-CoV-2 strains in Iran reveals potential contribution of co-infection with and recombination between different strains to the emergence of new strains. Virology. 2021.
35. Niyyati M, Lorenzo-Morales J, et al. Isolation and genotyping of potentially pathogenic Acanthamoeba strains from dust sources in Iran. Trans R Soc Trop Med Hyg. 2009;103(4):425-7.
36. Lasjerdi Z, Niyyati M, Haghighi A, et al. Potentially pathogenic free-living amoebae isolated from hospital wards with immunodeficient patients in Tehran, Iran. Parasitol Res. 2011;109(3):575-80.
37. Niyyati M, Lorenzo-Morales J, Rezaie S, et al. Genotyping of Acanthamoeba isolates from clinical and environmental specimens in Iran. Exp Parasitol 2009;121(3):242-5.
38. Alsam S, Jeong SR, Sissons J, Dudley R, Kim KS, Khan NA. Escherichia coli interactions with Acanthamoeba: a symbiosis with environmental and clinical implications. J Med Microbiol. 2006;55(6):689-94.
39. Rosenberg K, Bertaux J, Krome K, Hartmann A, Scheu S, Bonkowski M. Soil amoebae rapidly change bacterial community composition in the rhizosphere of Arabidopsis thaliana. ISME J. 2009;3(6):675-84.
40. Iqbal J, Siddiqui R, Khan NA. Acanthamoeba and bacteria produce antimicrobials to target their counterpart. Parasites & Vectors. 2014;7(1):1-6.
41. de Souza TK, Soares SS, Benitez LB, Rott MB. Interaction between methicillin-resistant Staphylococcus aureus (MRSA) and Acanthamoeba polyphaga. Curr Microbiol. 2017;74(5):541-9.
42. Elsafawy F, Wassef R, Amin N. Can endemic parasitic diseases and/or vectors play a role in the COVID-19 pandemic? Parasitologists United Journal. 2021;14(1):7-14.
43. Diesend J, Kruse J, Hagdorn M, Hammann C. Amoebae, giant viruses, and virophages make up a complex, multilayered threesome. Front Cell Infect Microbiol. 2018;7:527.
44. Legendre M, Lartigue A, Bertaux I, et al. In-depth study of Mollivirus sibericum, a new 30,000-y-old giant virus infecting Acanthamoeba. Proc Natl Acad Sci U S A. 2015;112(38):E5327-E35.
45. Colson P, Pagnier I, Yoosuf N, Fournous G, La Scola B, Raoul D. “Marseilleviridae”, a new family of giant viruses infecting amoebae. Arch Virol. 2013;158(4):915-20.
46. Maruyama F, Ueki S. Evolution and phylogeny of large DNA viruses, Mimiviridae and Phycodnaviridae including newly characterized Heterosigma akashiwo virus. Front Microbiol. 2016;7:1942.
47. Zauberman N, Mutsafi Y, Halevy DB, et al. Distinct DNA exit and packaging portals in the virus Acanthamoeba polyphaga mimivirus. PLoS Biol 2008;6(5):e114.
48. La Scola B, Audic S, Robert C, et al. A giant virus in amoebae. Science. 2003;299(5615):2033.
49. Seltzner CA, Ferek JD, Thoden JB, Holden HM. Characterization of an aminotransferase from Acanthamoeba polyphaga Mimivirus. Protein Sci. 2021; 30(9):1882-1894.
50. Hasöksüz M, Kılıç S, Saraç F. Coronaviruses and sars-cov-2. Turk J Med Sci. 2020;50(S1-1):549-56.
51. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020;26(8):1200-4.
52. Sakurai A, Sasaki T, Kato S, Hayashi M, Tsuzuki S-i, Ishihara T, et al. Natural history of asymptomatic SARS-CoV-2 infection. N Engl J Med. 2020;383(9):885-6.
53. Siddiqui R, Ahmed Khan N. Centralized air-conditioning and transmission of novel coronavirus. Pathog Glob Health. 2020;114(5):228-9.
54. Hsueh T-Y, Gibson KE. Interactions between human norovirus surrogates and Acanthamoeba spp. Appl Environ Microbiol. 2015;81(12):4005-13.
55. de Lacerda AG, Lira M. Acanthamoeba keratitis: A review of biology, pathophysiology and epidemiology. Ophthalmic Physiol Opt. 2021;41(1):116-35.