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The Role of *Acanthamoeba* in Preserving Ancient Viruses and Their Potential Spread Due to Climate Change

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Abstract Climate change is a multifaceted crisis that extends far beyond rising temperatures, posing a significant threat to both biological and ecological balance. One of the most concerning aspects of this crisis is the accelerated thawing of glaciers that have remained frozen for thousands of years enabling the reintroduction of microorganisms that have remained isolated for millennia into modern ecosystems (1). During this thawing process, particularly in permafrost layers, samples have revealed the presence of ancient giant viruses, such as *Mollivirus sibericum* and *Pithovirus sibericum*, alongside remnants of the 1918 Spanish flu-causing influenza virus (2, 3). These findings are not only of historical significance but also highlight the potential for novel biological threats to modern societies. One of the most critical agents in the environmental circulation of these viruses is *Acanthamoeba*. This free-living amoebae protects giant viruses by engulfing them through phagocytosis and facilitating their transmission into various environments. Moreover, the remarkable resilience of *Acanthamoeba* in extreme environmental conditions positions it not only as a host but also as a biological vector. This characteristic directly links the release of ancient viruses to their potential activation and dissemination. This review explores the mechanisms by which giant viruses released through glacial melting are transported via *Acanthamoeba*, assesses the potential infectious risks emerging in the context of climate change, and examines the broader consequences of this process. Additionally, the potential protective role of virophages in mitigating such risks is evaluated.

Keywords Giant viruses · *Acanthamoeba* · Virophages · Climate change · One health

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INTRODUCTION

Climate change poses emerging threats to human health not only through temperature fluctuations and the intensification of extreme weather events but also through the release of ancient microorganisms previously trapped in glacial ice. These ancient viruses, which have remained frozen for hundreds of thousands of years, may reenter ecosystems through glacier melt and potentially introduce novel health risks (1, 2). Among the most significant vectors of these viruses are *Acanthamoeba* species free-living amoebae that are widespread in nature and act as microbial reservoirs. By engulfing ancient viral particles, *Acanthamoeba* can preserve them and facilitate their transfer into modern ecosystems. This phenomenon raises concerns regarding the emergence of new diseases that could directly affect human populations (4).

However, virophages small viruses that infect giant viruses offer a potential biological defense mechanism capable of disrupting this cycle. This review aims to explore the transmission of ancient viruses through *Acanthamoeba*, evaluate the associated health risks and possible diseases, and discuss the potential mitigating role of virophages.

Biology of *Acanthamoeba*: Morphological and Physiological Characteristics

Acanthamoeba is a genus of free-living amoebae that exhibits a complex life cycle and remarkable physiological adaptability. It exists in two primary morphological forms: the active, feeding trophozoite stage and the dormant, resilient cyst stage. These morphological adaptations allow the organism to survive in various environments and contribute to its global distribution. Trophozoites utilize pseudopodia for movement and generally feed on bacteria and small microorganisms through phagocytosis (5).

In the trophozoite stage, *Acanthamoeba* cells are irregular in shape and typically range from 15 to 45 µm in diameter. They possess distinct nuclei, vacuoles, and other cellular organelles. Their active metabolic state enables efficient energy production through the oxidative phosphorylation of glucose. Trophozoites are highly motile due to their pseudopodia, which enhances their potential to disseminate infections (4).

Under unfavorable environmental conditions,*Acanthamoeba* transitions from the trophozoite form to a highly resistant cyst form. Cysts are encased in a double-walled structure: the inner wall, or endocyst, is composed primarily of cellulose, whereas the outer wall, or ectocyst, consists mainly of proteins and lipids. This robust structure renders the cyst resistant to extreme heat, cold, desiccation, chemical agents, and radiation (6). This physiological transformation, illustrated in Figure 1, is critical to the organism's environmental adaptability and resembles the long-term survival strategies seen in spore-forming bacteria (7).

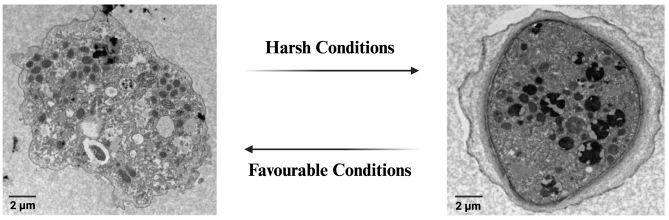

Figure 1. Transformation between trophozoite and cyst stages in the life cycle of Acanthamoeba spp. The diagram illustrates the morphological transition between the active trophozoite and resilient cyst forms under varying environmental conditions. (Created in BioRender. Baskale, EA. (2025) https://BioRender.com/rjzefs0)

The metabolism of *Acanthamoeba* is highly flexible, enabling energy production under aerobic and anaerobic conditions. In the presence of oxygen, ATP is produced through mitochondrial oxidative phosphorylation; in its absence, the switches to fermentation and glycolysis. This metabolic flexibility enables the organism to survive under a wide range of environmental conditions, including prolonged survival in hypoxic environments (7).

The primary feeding mechanism of *Acanthamoeba* is phagocytosis. It engulfs environmental food sources such as bacteria, viruses, algae, and small microorganisms using pseudopodia, internalizing them into food vacuoles where lysosomal enzymes perform digestion (5, 8).

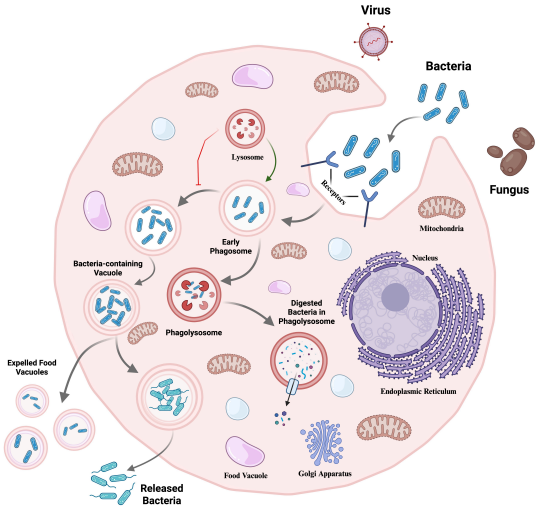

Figure 2. Schematic representation of the phagocytosis and digestion cycle in Acanthamoeba. This diagram illustrates how Acanthamoeba captures environmental microorganisms through pseudopodia, internalizes them into phagosomes, and processes them using lysosomal enzymes within digestive vacuoles. (Created in BioRender. Baskale, EA. (2025) https://BioRender.com/wwxxs4j)

In the context of climate change, the melting of glaciers and permafrost has led to the emergence of cold and nutrient-deprived environments, which underscore the physiological resilience of *Acanthamoeba*. The cyst form is particularly noteworthy for its ability to survive in frozen and arid conditions over extended periods. The ability of *Acanthamoeba* to survive in such conditions makes it a critical vector for both the preservation and dissemination of these ancient microbes (3).

Endosymbiosis refers to a symbiotic relationship where one organism lives inside another. *Acanthamoeba* species form endosymbiotic associations with bacteria, fungi, and giant viruses. These relationships offer protective advantages for microorganisms seeking refuge from external stress. Through its phagocytic capacity, *Acanthamoeba* engulfs these microbes, and those capable of evading lysosomal digestion can survive within the amoeba's cytoplasm as endosymbionts (4, 9). Some bacteria and giant viruses can even replicate within this intracellular environment or remain protected in cyst form until conditions become favorable again. Once inside the *Acanthamoeba* host, these viruses hijack the cell, transforming it into a "viral factory" for replication. Specialized replication zones are formed within the host cytoplasm, from which hundreds of new viral particles are synthesized (10).

Giant viruses such as *Marsuvirus*, *Pandoravirus*, *Pithovirus*, and *Mollivirus* exhibit a strong ecological and evolutionary association with *Acanthamoeba*, as they are capable of replicating within this host. This relationship facilitates viral replication and environmental protection for viruses against external stressors. In addition to its relationship with viruses, *Acanthamoeba* forms endosymbiotic associations with certain bacterial species. For instance, *Parachlamydia* can inhabit amoeba such as *Acanthamoeba*, providing protection against viral infection processes. These bacteria can inhibit viral replication, preventing host cell death, and establishing mutualistic symbiosis (11). An important consequence of these symbiotic structures is the environmental transport and preservation of microorganisms through *Acanthamoeba*. This transportation may play a significant role in the environmental spread of ancient pathogenic viruses released from melting glaciers. As a result, the probability of ancient viruses integrating into modern ecosystems increases not only through spatial diffusion but also across time scales, as organisms once frozen for millennia are reintroduced into the environment (3).

Ecological Distribution of *Acanthamoeba*

Acanthamoeba species are free-living protozoa with a broad ecological distribution. They are commonly found in various environmental settings, including soil, water, air, dust, and organic matter-rich habitats (4). Their high prevalence in environments rich in organic materials underscores their adaptability and ability to influence microbial community structure. By establishing symbiotic relationships with bacteria, *Acanthamoeba* plays a critical role in shaping the population dynamics of environmental microbial communities. These interactions are essential for maintaining ecosystem balance and regulating the spread of pathogens (9). In regions withof high biodiversity, *Acanthamoeba* can serve as a reservoir for pathogenic microorganisms. The amoebae form both symbiotic and endosymbiotic relationships with bacteria, fungi, and especially giant viruses (16). For example, *Legionella pneumophila* can replicate within *Acanthamoeba* cells, gaining protection and enabling dissemination across wide environmental areas, such as water systems, thereby posing a potential health risk (9). Different genotypes of *Acanthamoeba* evolved to adapt to various ecological conditions. Among these, the T4 genotype is frequently identified in clinical samples due to its high pathogenic potential and robust environmental resilience (5). In response to environmental stressors, *Acanthamoeba* can actively feed and move as a trophozoite, but under adverse conditions, it transitions into a resilient cyst form, enhancing long-term survival (4, 17).

Recent studies have uncovered complex ecological and evolutionary relationships between *Acanthamoeba* and giant viruses. Large DNA viruses such as *Mimivirus*, *Marseillevirus*, *Pithovirus*, and *Mollivirus* enter amoeba cells through phagocytosis and replicate within them. *Mimivirus* utilizes the host cytoplasm to establish viral factories after internalization, whereas *Marseillevirus* enhances its transmission potential through capsid and vesicle structures that confer environmental resistance (18). These evolutionary associations are further influenced by bacterial symbionts that suppress giant virus infections. For instance, *Parachlamydia* lives symbiotically within *Acanthamoeba*, inhibiting giant virus replication and prolonging the host cell's survival by preventing viral entry (11).

Additionally, ancient viruses, such as *Mollivirus sibericum*, isolated from 30,000-year-old permafrost samples, have been shown to infect *Acanthamoeba* and remain viable under laboratory conditions. This discovery, made by Legendre and colleagues in 2015, supports the notion that ancient viruses could be reintroduced into modern ecosystems because of climate change and permafrost thawing (3). The transport of these viruses via *Acanthamoeba* represents a substantial risk for ecological balance and public health. Furthermore, if reactivated, these long-dormant viruses may undergo mutations that could enhance their pathogenicity.

Acanthamoeba species are globally distributed protozoans that inhabit a wide range of ecological niches. They have been isolated from diverse environments, including soil, freshwater and marine systems, air, dust, hospital equipment, air conditioning systems, contact lenses, and lens cases. Their environmental persistence is largely due to their remarkable resistance to stress, particularly in their cyst form (19). This high environmental resilience significantly

enhances their survival and dispersal capacity (6). In recent years, increased human mobility, poor hygiene, and the widespread use of contact lenses have contributed to the broader geographic spread and human exposure to these amoebae. Inadequate disinfection of contact lenses significantly increases the risk of infection and promotes their dissemination (20).

However, one of the most critical modern drivers of *Acanthamoeba* dispersal is climate change. Environmental phenomena, such as glacier melt and permafrost thaw, have enabled the reactivation of ancient microorganisms and viruses. Studies show that permafrost can preserve viruses for thousands of years, and with thawing, these pathogens may be rereleased into modern ecosystems (3). In this context, *Acanthamoeba* species may serve as key vectors in the transport and dissemination of these ancient viruses. The emergence of such microorganisms could introduce potential epidemic risks to current ecosystems.

Recent research suggests that *Acanthamoeba* may not only serve as a host but also act as a biological vector that facilitates the reactivation of ancient viruses (21). For example, giant viruses such as *Mimivirus*, *Mollivirus*, and *Pithovirus*, which have been shown to remain viable in 30,000-year-old permafrost layers, can infect *Acanthamoeba* cells and replicate within them under laboratory conditions (3, 21). This presents not only an ecological threat but also a potential global risk of new zoonotic infections or future pandemics (16, 21).

The transport of ancient viruses by *Acanthamoeba* represents a mechanism with global transmission potential. These amoebae are commonly found in human-associated environments and have extensive ecological reach (19). As glaciers melt and permafrost thaws, ancient viruses can interact with environmentally ubiquitous *Acanthamoeba* and be carried across wide geographic ranges (11, 21). This suggests a strong correlation between the environmental spread of *Acanthamoeba* and the climatic processes driving permafrost thaw.

In 2023, Alempic et al. demonstrated that ancient viruses isolated from Siberian permafrost could be reactivated in vitro using *Acanthamoeba* cells (21). These findings underscore the importance of understanding *Acanthamoeba* ecology and limiting its spread to prevent potential future outbreaks. Further research should focus on the ecological distribution, resistance mechanisms, and interactions between *Acanthamoeba* and ancient viruses. Such knowledge holds strategic importance for global health security.

Diseases Caused by Acanthamoeba Species

Acanthamoeba species are recognized as potential pathogens of critical concern for human health. Infections caused by these amoebae can lead to severe illnesses, particularly in immunocompromised individuals. Although transmission is most frequently associated with contact lens use, it may also occur through natural sources such as soil, freshwater, and stagnant water. Poor hygiene practices significantly increase the risk of transmission, especially via contaminated contact lenses (20). The two primary diseases associated with *Acanthamoeba* are *Acanthamoeba* keratitis (AK) and Granulomatous AmebicAmoebic Encephalitis (GAE) (20).

***Acanthamoeba* keratitis** is an infectious disease of the cornea that can result in severe vision loss. Although it has a low incidence rate, it is often resistant to treatment due to difficulties in early diagnosis and the presence of resilient cyst forms of the amoeba. ThisThe condition is more frequently observed in contact lens users and individuals living under unhygienic conditions (6).

Granulomatous amoebic encephalitis (GAE) is a far rarer but significantly more lethal disease. GAE affects the central nervous system primarily in immunocompromised individuals and is associated with high mortality rates. The disease typically begins with symptoms resembling chronic meningitis and gradually progresses to include neurological deficits, coma, and death (4).

Key Ancient Viruses Associated with Acanthamoeba

Mimivirus: *Mimivirus* genus of giant viruses belonging to the *Mimiviridae*; family, was first discovered accidentally in 1992 within the amoeba *Acanthamoeba polyphaga*, where it was found to replicate (41). With a giant DNA genome of approximately 1.2 megabases (Mb), organized within a protein shell, the viral genome is in a transcription-ready state upon entering the host cytoplasm, allowing for immediate protein production. This efficient expression mechanism makes *Mimivirus* a unique model for studying viral evolution and genome organization (42).

Pandoravirus: *Pandoraviruses*, giant viruses with some of the largest known viral genomes reaching up to 2.8 Mb, were first identified in 2013. These viruses replicate exclusively within *Acanthamoeba castellanii* and are morphologically amphora-shaped (13). Genetic manipulation using CRISPR/Cas9 systems has enabled in-depth investigation of their essential genes and evolutionary pathways (14).

Mollivirus sibericum: *Mollivirus sibericum* is the fourth ancient giant virus isolated from Siberian permafrost, discovered in 2003 (43). This spherical DNA virus, with a diameter of 500–600 nanometers (0.5–0.6 µm), replicates within *Acanthamoeba castellanii* (3). Unlike classical giant viruses that form cytoplasmic viral factories, *Mollivirus* performs replication within the host cell nucleus. This distinct feature makes it an important subject for studying alternative viral adaptation strategies (3).

Pithovirus sibericum: *Pithovirus sibericum* is a double-stranded DNA virus that infects amoebae. It was first described in 2014 in a viable sample extracted from a 30,000-year-old Siberian permafrost ice core (44). Approximately 50% larger than previously known giant viruses, *Pithovirus* is characterized by massive viral particles up to 1.5 µm in length. It induces structural alterations

within *Acanthamoeba* cells, facilitating the morphological analysis of virus-host interactions (13).

Atmospheric Dispersal of Ancient Microorganisms via *Acanthamoeba* and Potential Global Health Risks

One of the most dominant forms of atmospheric aerosols critical to Earth's ecosystems is mineral dust particles—commonly known as desert dust. These particles rise from arid regions and travel long distances through the upper layers of the atmosphere. While larger particles tend to settle near their source, smaller particles can traverse thousands of kilometers. The increasing presence of such particles in the atmosphere is primarily attributed to climate change-driven desertification and drought conditions (22). In addition, primary biological aerosol particles (PBAPs), which remain suspended in the atmosphere, comprise bacteria, viruses, and fragments of microbial cells (23). These biologically active aerosols, capable of long-range transport and cloud condensation nucleation, may enhance the environmental mobility of pathogens, influencing both climate and public health. Ancient microorganisms and viruses released through glacial melting have been transported over long distances as biological aerosols via *Acanthamoeba*, which is widely used as a model for studying ancient viruses (24). In this process, *Acanthamoeba*'s durable cyst structure may protect viruses under atmospheric conditions, enabling their survival and distribution into modern ecosystems. As such, the role of *Acanthamoeba* in the atmospheric mobility of ancient viruses may pose significant global health risks (24).

Interaction of Acanthamoeba with Ancient Viruses in the Context of Climate Change: Potential for Novel Diseases

As climate change accelerates glacial melting, ancient microorganisms, including giant viruses preserved beneath ice, are re-entering modern ecosystems. These viruses, characterized by their enormous size and complex genomes, have drawn significant scientific interest. However, their capacity to be carried and activated through amoebic hosts such as *Acanthamoeba* also raises considerable concerns (3). Given the ecological ubiquity of *Acanthamoeba* and its role as a host, it is anticipated that it may serve as a vector, facilitating the dissemination of ancient viruses into modern environments and across human populations (16). Notable examples include *Mimivirus*, *Pithovirus*, and *Mollivirus*, which have been isolated from permafrost and successfully reactivated in modern culture systems, as mentioned before, including those involving algae and bacteria, such as *E. coli* (25).

Within *Acanthamoeba*, these viruses engage in complex molecular interactions, creating large viral factories that lead to massive replication. This process culminates in host cell lysis and widespread viral release. This cycle not only accelerates transmission but also promotes mutations that may enhance viral infectivity (26). Recent genomic studies have revealed horizontal gene transfer events between *Acanthamoeba* and giant viruses. Certain viral genes have been integrated into the amoeba genome, resembling lysogenic cycles observed in bacteriophages, where viral DNA is incorporated into the host's genome and passed on to subsequent generations (27). These horizontal gene transfers may facilitate the emergence of new pathogenic traits, raising concerns about future outbreaks (12).

The molecular structure of these viruses may enable the synthesis of proteins unrecognizable by the human immune system. Since modern human populations have never been exposed to these ancient viruses, immune responses may be inadequate or absent. This increases the likelihood of novel infections and underscores the threat posed by these re-emerging biological agents (16).

Risk of Ancient Virus Infections in Humans via Acanthamoeba

To date, mimiviruses have been associated with pneumonia, whereas marseilleviruses have been linked to adenitis and lymphoma (28, 29). One strain of mimivirus, the *Shan virus*, was isolated from the stool of a patient presenting with pneumonia and diarrhea (30).

Although gastrointestinal symptoms have not yet been conclusively linked to *Acanthamoeba*-associated giant viruses, no dedicated clinical studies have specifically addressed this potential correlation. However, mimivirus-related sequences have been detected in fecal samples from both healthy individuals and patients suffering from pneumonia (30), diarrhea (31), and inflammatory bowel disease (32). The presence of mimiviruses in fecal matter may be secondary to respiratory tract infections, as has been observed with other microorganisms, such as *Mycobacterium spp*. (33). To support this hypothesis, mimiviruses have been isolated from bronchoalveolar lavage fluid and feces of two patients with pneumonia (30).

Additionally, mimiviruses have been shown to infect human macrophages, enter *Acanthamoeba* via phagocytosis in a similar fashion, and persist within cells in a manner comparable to the lysogenic phase of bacteriophages. Furthermore, they have been observed to replicate in peripheral blood mononuclear cells (35–37). In an experimental model, cellular infiltrates composed of mononuclear leukocytes, macrophages, and lymphocytes were observed, along with widespread alveolar damage following intracardiac inoculation of mimiviruses (38). Collectively, these findings suggest that mimiviruses may infect intestinal macrophages or trigger local inflammation in the human gut. Regarding Marseilleviruses, their presence has been identified through culture isolation and high-throughput sequencing of human fecal samples, as well as via PCR, fluorescence in situ hybridization (FISH), immunofluorescence, and immunohistochemistry from blood and lymph node tissues (39). A *Marseillevirus* isolated from human blood was still detectable within Jurkat cells, a human T lymphocyte immortalized cell line, even on day 21 post-inoculation,

although without active replication (39, 40). In 2018, *Mimivirus* and *Marseillevirus* were detected in human skin lesion samples in the USA using metagenomic sequencing (63). In 2019, a global virome-wide study identified virophages in human fecal samples collected from multiple regions (64). In Iran, *Mimivirus* was found in tuberculosis-suspected patients' samples through nested PCR and real-time PCR techniques in 2022 (62). In 2024, metagenomic sequencing revealed the presence of *Mimivirus* in upper respiratory tract samples of COVID-19 patients, suggesting a potential interaction between *Mimivirus* and SARS-CoV-2 infection (65). In 2024, a study in Poland found *Mimivirus, Marseillevirus*, and *Pandoravirus* in human placenta samples from pregnancies with and without fetal growth restriction using virome and proteomic analysis (66). (Table1).

Table 1. Cases associated with the presence of giant viruses in human clinical samples (2008-2024).

Year	Virus(es)	Country	Population / Sample Info	Detection Method	References
2008	*Mimivirus*	Australia / USA	12 children with diarrhea	Metagenomic sequencing	(31)
2012	*Senegal virus, Mimivirus*	Senegal	20-year-old healthy individual	Amoebal culture	(67)
2012	*Marseillevirus, Mimivirus*	Senegal	20-year-old healthy individual	Next-generation sequencing	(30, 67)
2013*	*Shan virus, Mimivirus*	Tunisia	17-year-old patient with pneumonia	Amoebal culture	(30)
2013	*Virophage*	N/A	Human gut samples	Metagenomic sequencing	(68)
2013	*Mimivirus*	Belgium	Fossilized feces from the 14th century	Metagenomic sequencing	(69)
2015	*Mimivirus*	United Kingdom / USA	Patients with inflammatory bowel disease and controls	Metagenomic sequencing	(70)
2016	*Mimivirus, Marseillevirus*	N/A	Human Microbiome Project samples	Metagenomic sequencing	(71)
2018	*Mimivirus, Marseillevirus*	USA	Human skin lesions	Metagenomic sequencing	(63)
2019	*Virophage*	Global (Human gut samples from multiple regions)	Human fecal samples (virome-wide analysis)	Metagenomic sequencing	(64)
2022**	*Mimivirus*	Iran	Tuberculosis-suspected patient samples	Nested-polymerase chain reaction (PCR) and Real-time PCR	(62)
2024	*Mimivirus*	Global (SARS-CoV-2 context)	Upper respiratory tract samples of COVID-19 patients	Metagenomic sequencing	(65)
2024	*Mimivirus, Marseillevirus, Pandoravirus*	Poland	Human placenta samples (Pregnancies with and without fetal growth restriction)	Virome and proteomic analysis	(66)

* Samples for the 2013 study were collected between 2010 and 2012.
** Samples for the 2022 study were collected between 2013 and 2017.

In addition to mimiviruses and marseilleviruses, the main evidence supporting the existence and potential pathogenic role of giant viruses and virophages in humans is illustrated in a timeline covering key findings from 2003 to 2016. This schema, originally compiled by Colson et al, has been adapted and presented in Figure 3 (28).

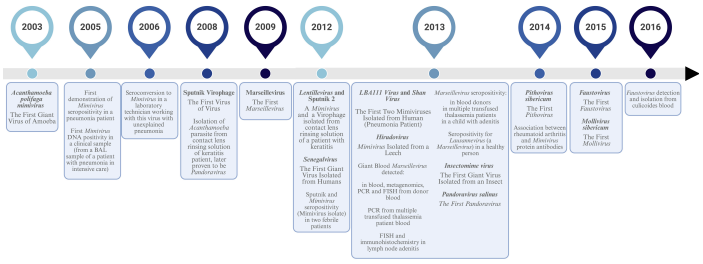

Figure 3. Chronological scheme of the major findings supporting the existence and possible pathogenic role of giant viruses and virophages in humans, 2003–2016 [28]. (Created in BioRender. Baskale, EA. (2025) https://BioRender.com/yfry6lb).

Virophages Infecting Ancient Viruses and Their Relationship With *Acanthamoeba*

Virophages and Their Hosts

Virophages are small viruses that infect giant viruses. They can disrupt the replication cycle of giant viruses, such as *Mimivirus*, and inhibit their ability to reproduce efficiently within host cells (45). Most known virophages have been found in association with free-living amoebae of the *Acanthamoeba* genus, as these amoebae are the primary hosts of giant viruses (45).

Interactions Between Virophages, Giant Viruses, and Acanthamoeba

Certain virophages that limit the replication of giant viruses (e.g., *Mimivirus* and *Pandoravirus*) inside *Acanthamoeba* do so by interfering with the giant virus genome. This suppression not only inhibits viral propagation but also reduces the damage inflicted on the host amoeba. The complex interplay between virophages and giant viruses within *Acanthamoeba* plays a significant role in shaping the evolutionary trajectory of these microorganisms.

In addition to these viral interactions, some bacteria that infect *Acanthamoeba* have also been shown to interact with giant viruses. These interactions can facilitate the horizontal transfer of antibiotic resistance genes across species, potentially altering the ecological balance of microbial pathogens and contributing to the spread of antibiotic-resistant bacteria (46).

Although the direct link between virophages, giant viruses, and human health remains poorly understood, their pathogenic potential in immunocompromised individuals is still under investigation (46). The biological characteristics of virophages and their effects on viral replication have been explored in detail, notably through studies of the *Sputnik* virophage, which infects the *Acanthamoeba polyphaga mimivirus* (APMV). This virophage-virus interaction reveals complex dynamics within the viral ecosystem, suggesting multilayered regulatory mechanisms within amoebic hosts (47). These interactions are visually summarized in Figure 4, which illustrates the interplay between virophages, giant viruses, and *Acanthamoeba* at the cellular level.

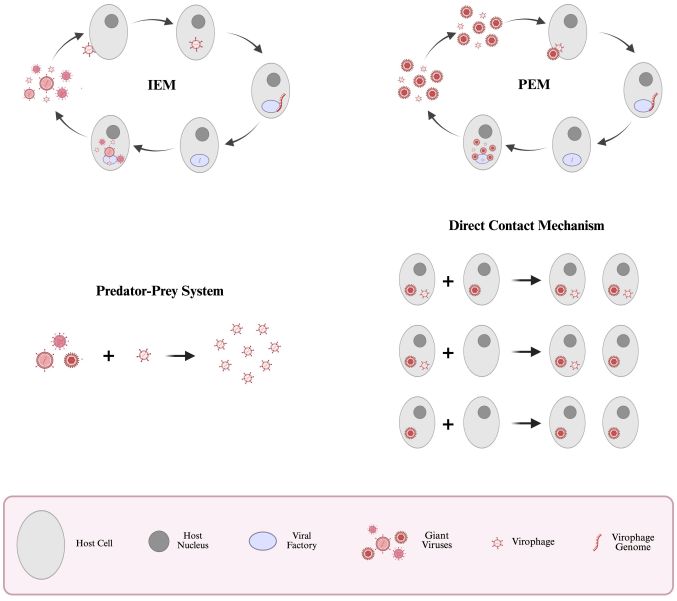

Figure 4. Virophage and ancient virus co-infection life cycle. IEM: Independent Entry Mode, PEM: Paired Entry Mode (Created in BioRender. Baskale, EA. (2025) https://BioRender.com/kh4guc2).

Wilhelm et al. (2017) provided a detailed account of how giant viruses infect small eukaryotes and how virophages interfere with these processes (48). The first isolated virophage, named Sputnik, replicates within the viral factory established by *Mamavirus* inside *Acanthamoeba castellanii*. Thus, Sputnik can only reproduce within *A. castellanii* cells co-infected with Mamavirus. Co-incubation of *Sputnik* and *Mamavirus* reduced infectious Mamavirus particle titers by approximately 70%, thereby increasing the survival rate of *A. castellanii* (29). Similar virophages have also been found to infect other giant viruses (49–52).

Co-Infection Cycles between Virophages and Ancient Viruses

Viruses are often classified as non-living entities because they are acellular, cannot maintain homeostasis, do not grow, and lack their own energy production systems. However, the discovery of viruses that infect other viruses has challenged this definition and strengthened the argument that viruses may exhibit the properties of living organisms (53, 54).

Some mimivirus clades appear to have developed a CRISPR-Cas–like defense system known as Mimivirus Virophage Resistance Element (MIMIVIRE) to counter virophage infections (55). Interestingly, a series of genes homologous to those found in the MIMIVIRE system have also been identified in other giant viruses, suggesting that such defense mechanisms may not be exclusive to mimiviruses (56, 57). Despite supporting findings from various studies, many aspects of these systems remain unclear and require further investigation (45).

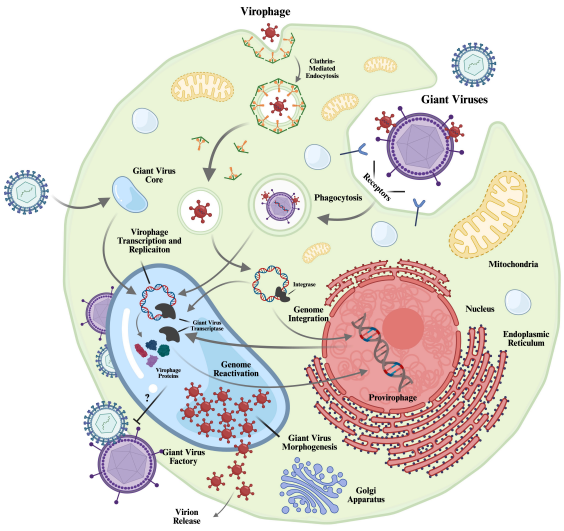

Figure 5. Proposed ancient virus and virophage infection cycle in an eukaryotic host cell (Acanthamoeba). (Created in BioRender. Baskale, EA. (2025) https://BioRender.com/mj8cd57).

Some ancient virus capsids (e.g., *Mimivirus*) are covered with fibers that allow the co-entry of virophages (e.g., *Sputnik*) through phagocytosis. Other virophages, such as *Mavirus*, enter cells via receptor-mediated endocytosis. After capsid uncoating and fusion of the internal membrane with either the phagosomal or cytoplasmic membrane, the viral core is released into the cytoplasm, where it transforms into a viral factory. The virophage genome is targeted to the factory, where it is activated during the late stage of infection by the transcription machinery encoded by the ancient virus. Replication of the virophage genome is catalyzed by virophage-

encoded DNA polymerases and helicases. Virophage particles assemble within or near the viral factory and are subsequently released upon host cell lysis. The proteins produced during virophage replication and transcription can inhibit the production of the ancient virus by interfering with genome-level replication pathways. Alternatively, the *Mavirus* genome can integrate into the nuclear host genome independently of a giant virus. Although the provirophage genes remain transcriptionally silent, they can be activated upon infection by a compatible giant virus, leading to the production of virophage particles within the ancient virus factory (58). (Figure 5).

Impact of Virophages on Biodiversity of Viral Ecosystems and Potential Use for Prevention of Global Health Threats

Acanthamoeba species facilitate the spread of these agents into new ecosystems because they can harbor a variety of microorganisms, including ancient viruses released through glacial melting. Consequently, *Acanthamoeba* can act as a reservoir for pathogens capable of causing infections in humans and animals. Virophages, by controlling the population dynamics of giant viruses, play a critical role in shaping microbial ecosystems. Particularly in aquatic environments, virophages act as balancing factors by regulating infections caused by giant viruses. (59).

In addition to ancient viruses, *Acanthamoeba* species are capable of harboring and transmitting various pathogens, including orthopoxviruses, such as the smallpox virus, which was previously eradicated through vaccination. This raises concerns about the ease with which viruses could be transmitted across hosts, potentially leading to new global health threats (59). Currently, there is speculation that *Acanthamoeba* might aid in the survival and transmission of emerging threats, such as the monkeypox virus. Primary hosts like rodents and incidental hosts such as monkeys and dogs can shed the monkeypox virus in bodily secretions. Environmental *Acanthamoeba* may harbor the virus, facilitating its transmission to humans. Once in humans, further environmental contamination could occur, with newly released viruses being reabsorbed by *Acanthamoeba*, acting as a Trojan horse and accelerating widespread community transmission (59). (Figure 6).

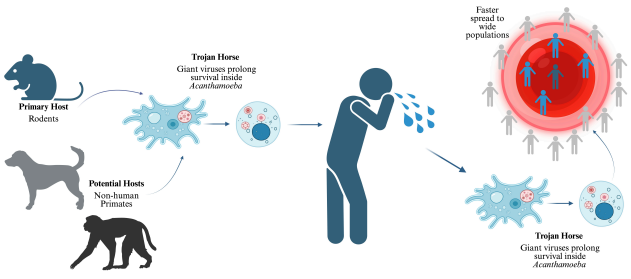

Figure 6. Transmission of ancient viruses to susceptible hosts via Acanthamoeba (Created in BioRender. Baskale, EA. (2025) https://BioRender.com/lix38zt).

Ancient viruses, following a similar pathway, may survive environmental conditions during their release into oceans after glacial melting triggered by global warming, facilitating long-distance dispersal and host-switching events. Consequently, it must be considered a legitimate possibility that such viruses could infect humans and broader populations, potentially leading to large-scale global pandemics.

CONCLUSIONS

The discovery of *Mimivirus* originating from *Acanthamoeba* not only heralded the emergence of a new field of scientific research but also profoundly shaped its subsequent evolution. Giant viruses have now become one of the most critical subjects in evolutionary, ecological, and biotechnological studies. Moreover, large-scale investigations aimed at identifying new virus host systems—ranging from classical culture-based methods to advanced bioinformatics linking viruses with their hosts—are expected to soon provide a more comprehensive database concerning the fundamental properties of these novel viral particles (60).

In the future, the study of giant viruses is anticipated to illuminate not only virus–host interactions but also fundamental evolutionary processes, including the natural rates of transduction and horizontal gene transfer (60).

Acanthamoeba species, by harboring a variety of microorganisms and/or pathogens—including ancient viruses released from melting glaciers—facilitate their dissemination into ecosystems and enable survival under harsh environmental conditions. Consequently, they may serve as reservoirs causing infections in humans and animals. This highlights the importance of the "One Health" concept, which emphasizes the interconnectedness of humans, animal, and environmental health. Numerous studies on various metabolic, reproductive, and survival systems in eukaryotic species have been conducted using *Acanthamoeba* as a model organism. These amoebae are unique due to their invasive strategies, phagocytosis-based target capture mechanisms, and the ability to phenotypically adapt to diverse environmental conditions (61).

All these approaches pave the way for the development of agents that could be effective against both viral pathogens and Acanthamoeba hosts. Understanding the molecular foundations of these interactions, such as variations in gene expression or receptor configurations utilized by microorganisms, can promote the One Health approach and shed light on strategies to manage pathogenicity and drug resistance.

In this context, it is suggested that both targeting *Acanthamoeba* amoebae and assessing the usability of virophages that infect viruses could be promising avenues for infection control strategies. Prospective research and innovative molecular studies are still needed to better understand these

interactions and evaluate the effectiveness of such targeting strategies.

In conclusion, the potential for ancient giant viruses released through climate change to be transmitted via *Acanthamoeba* and disseminated into the modern world poses significant new pandemic threats. This risk is further complicated by the viruses' high genetic diversity, horizontal gene transfer capacity, and potential for host-independent replication. In the coming years, an intensified focus on molecular, genetic, and epidemiological studies related to these viruses will be essential for better understanding these emerging health threats and for developing effective countermeasures. Future research must prioritize the development of strategic protection measures against such novel health risks.

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