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SYSTEMATIC REVIEW OF THE EFFECTS OF ACANTHAMOEBA POLYPHAGA MIMIVIRUS (APMV) INFECTION ON VIRULENCE OF ACANTHAMOEBA POLYPHAGA (AP)

By

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Abstract

The interactions between *Acanthamoeba polyphagan* (AP) and *Acanthamoeba polyphaga mimivirus* (APMV) play a crucial role in their virulence and adaptability. This systematic review synthesises findings from 41 studies published between 2003 and 2024 to evaluate how APMV infection impacts these organisms. The review highlights key genetic and molecular changes, including alterations in gene expression, phagocytosis, and encystment, alongside the influence of environmental factors. APMV infection significantly affects virulence, driven by complex symbiotic relationships. The review gave a clearer understanding of AP & APMV interactions, with implications for microbial ecology and disease management.

Key words: *Acanthamoeba polyphaga mimivirus*, *A. polyphaga*, Virulence, Review

Introduction

Acanthamoeba spp., are commonly found in lakes, swimming pools, tap water, and heating and air condition unit, including *A. culbertsoni*, *A. polyphaga*, *A. castellanii*, *A. astronyxis*, *A. hatchetti*, *A. rhysodes*, *A. divionensis*, *A. lugdunensis*, and *A. lenticulata* are implemented in the zoonotic disease. They are considered the most abundant free-living amoebae in nature, and their various species can be acquired from dust, soil, all types of water, and various medical devices or supplies (CDC, 2019). *Acanthamoeba polyphaga* (AP) is usually exposed to a wide range of organisms, chemicals, and extreme conditions, prompting them to engage in a broad spectrum of stochastic processes, causing a robust capacity to interact successfully with their environment and survive (Fanselow *et al,* 2021). AP's virulence factors include its unique ability to host a wide range of organisms from all domains of life, as well as viruses in symbiosis (Rayamajhee *et al,* 2021). AP endosymbiont viruses include human viruses like adenovirus (Staggemeier *et al,* 2016), giant viruses like *A. polyphaga mimivirus* (APMV) and virophages like Sputnik 2 (Cohen *et al,* 2011). Further, AP can prompt their growth under the extreme conditions (Lamrabet *et al,* 2012).

APMV is a non-enveloped, icosahedral, double-stranded DNA (dsDNA) virus was first isolated in 1992 from a cooling tower during an investigation of a pneumonia outbreak in Bradford, United Kingdom. APMV resembles bacteria in particle size and genome and has a surface covered in fibres layered with a glycan, similar to the peptidoglycan layer found in bacterial cell walls (Rodrigues *et al,* 2015). Further characterisation of the virus revealed various unprecedented properties, such as the large size of its virion and its 1.2 Mbp genome, which are larger than all known viruses and even some bacteria (Raoult *et al,* 2004). The virus resembles bacteriophages due to its unique capsid vertex similar to that of tailed bacteriophages (Zauberman *et al,* 2008). The APMV shows similarities to eukaryotes in the containment of its genome within an internal envelope supported by fibres, akin to eukaryotic cell nucleus (Kuznetsov *et al,* 2010). The amoebic host range of APMV includes *Acanthamoeba polyphaga, A. castellanii, A. griffin* and *A. lenticulata* (Claverie *et al,* 2009). To date, 207 strains similar to APMV are identified, known as giant viruses and classified in order Imitravirales (Schoch *et al,* 2020).

The current systematic review aims to examine how APMV infection influences the

virulence of both Acanthamoeba polyphaga (AP) and APMV, focusing on their genetic and molecular interactions. The objectives of this review are to determine the types of studies used to investigate the effect of AP infection by APMV on their virulence; to identify the types of cultures employed to examine the effects of various ecological factors on the virulence of both organisms; to determine the virulence factors of both organisms affected by the infection and evaluate the impact on each virulence factor; and to assess the effects of symbiosis between AP, APMV, and the endosymbionts of AP on infection dynamics and the resulting changes in virulence. Accordingly, the study will provide a comprehensive synthesis of current findings and highlight the broader implications for future research.

Results

Electronic databases identified 1102 publications (Medline, 342, WoS, 131, Sciencedirect, 284, JSTOR 135, & Google Scholar, 210). Duplications eliminated 119 articles based on title and abstract were 983. Identification of 134 publications was okay for eligibility criteria, included 41 (Fig. 1).

Included studies: A total of 41 were analysed. Studies were examined for their type of study, cultures and media, and the effects of the infection on both organisms were analysed (Tab. 1).

Quality and risk of bias in individual studies: Risk of bias assessment was conducted for each of the 41 included studies using the NOS tool. All scored studies between 8 & 9 in the overall value reflected the strict selection criteria used (Fig. 2, Tab 2).

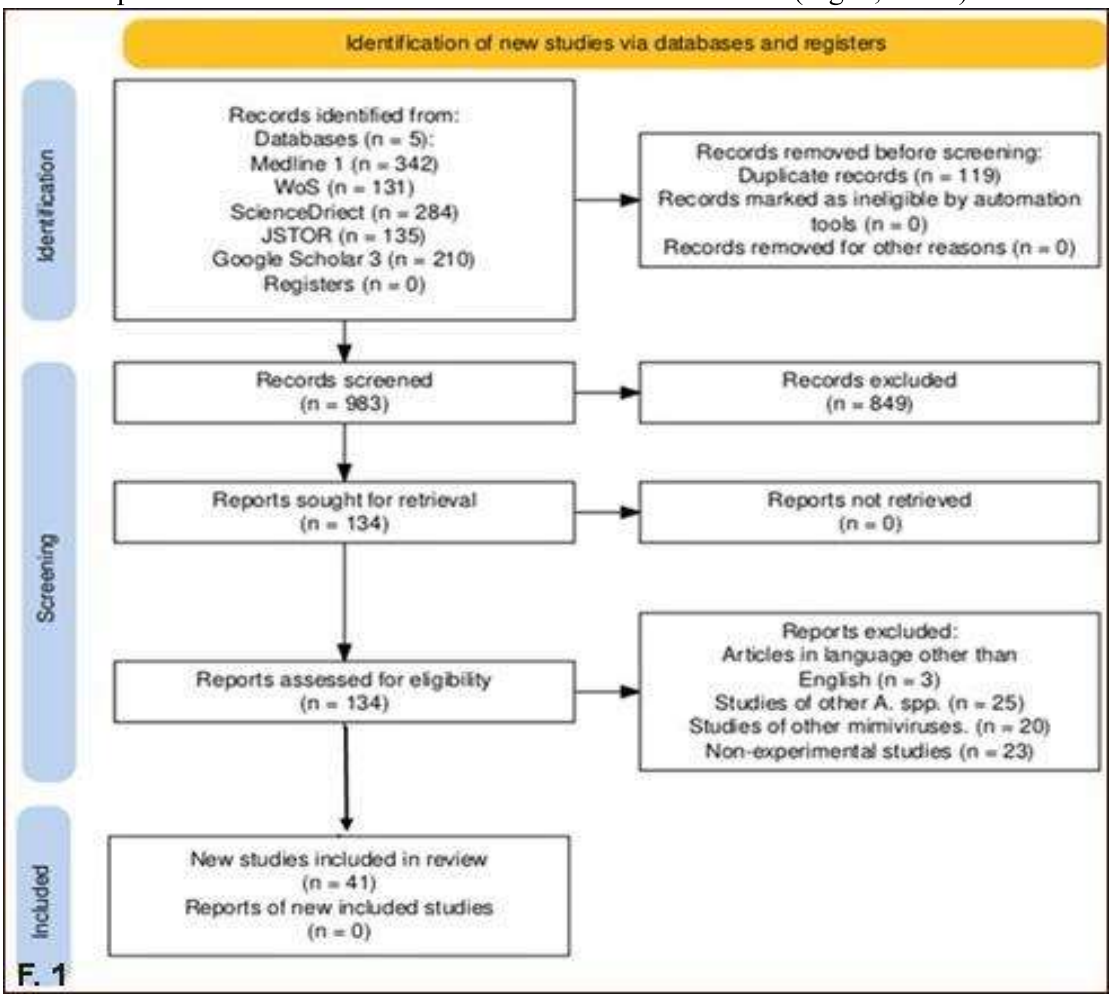

Fig 1: Flow chart of study selection according to PRISMA regulation.

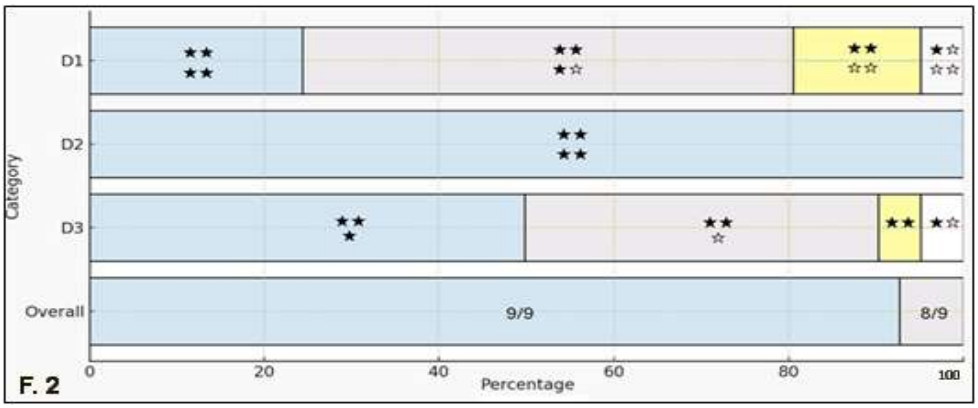

Fig 2: Bias of risk assessment by domain with Newcastle-Ottawa scale (NOS).

Systematic review and Discussion

This systematic review was conducted following the guidelines of the Preferred Reporting Items for Systematic Review and Meta-Analysis (Moher *et al,* 2009).

The primary research question that formed the basis of this systematic review was: How does the infection of AP by APMV influence the virulence and adaptability of both organisms under different environmental conditions? To answer the question, employed PICO framework; patient/population, intervention, comparison, outcome were used (Richardson *et al,* 1995). The population was limited to studies involving AP and APMV; the intervention was the infection of AP by APMV; the comparison considered different environmental conditions, such as variations in sterility, temperature, pH, nutrient availability; and the outcomes of interest were changes in the virulence and adaptability of both AP &APMV. These criteria guided the development of our search strategy, the selection of studies, data extraction, and the synthesis of findings to ensure a comprehensive evaluation of the existing evidence.

Eligibility: Inclusion criteria were original, experimental, peer-reviewed studies, available as full text, published in English, examining the interaction of APMV and AP in infections of AP as a primary or hyper host for APMV alone or with other endosymbionts. The exclusion criteria eliminated of all studies before 2003, on *Acanthamoeba* spp., laboratory protocols, scientific meeting, and reviews except for systematic ones and meta-analysis, conference abstracts.

Search strategy and data sources: Initially, a systematic search was implemented utilizing five electronic databases, Web of Science (WoS), PubMed (Medline), J. Storage (JSTOR), ScienceDirect and Google Scholar, to generate a dataset of published literature related to the study. The key terms used in the databases search were *Acanthamoeba polyphaga* and (giant virus, or mimivirus, or megavirus). The search was performed on 1st July 2024 and it was restricted to studies conducted between 2003 & 2024 in English.

Study selection and data extraction: This systematic review was done following evidence synthesis according to PRISMA guidelines, implementing eligibility criteria. All data generated by database search were uploaded to EndNote X9 (Philadelphia, USA). Software organised citations from databases, remove duplicates, and centralised on library of all relevant studies (The End-Note Team 2013). EPPI Reviewer 6 (version 6.15.4.: EPPI-Centre, Social Science Research Unit, UCL, London). Software also enabled coding and categorisation of study characteristics and outcomes (EPPI-Centre 2024). Flow diagram of process was done by PRISMA Flow Diagram tool (Haddaway *et al,* 2022).

Data items: Virulence factors affected by APMV infection of AP included phagocytosis, encystment, environmental sensing, mobility, gene transfer and extended survival in symbiosis.

Quality and risk of bias assessment in each studies: Newcastle-Ottawa Scale (NOS) was utilised to assess the potential risk of bias in all studies (Stang 2010). The tool was adjusted after cross-sectional guidelines and observational ones. An overall value was designated for each one based on fulfilment of subdomains of the three domains scale, using highly heterogeneous and thus meta-analysis was not performed.

Types of studies investigated the effect of AP infection by APMV on their virulence: Their analysed were categorised into three types to investigate the effect of APMV infection of AP on their virulence: molecular and genomic, cellular and in vitro, and ecological and symbiotic studies. Molecular and genomic research included transcriptomic and genomic analyses, comparative genomics, molecular characterisation and functional analysis and structural and biochemical studies. The cellular and in vitro category included cellular and morphological analysis, phagocytosis and host-pathogen interactions, and functional and in vitro studies. The ecological and symbiotic category included symbiosis and co-evolution, tripartite symbiosis, viral ecology and environmental as well as adaptation studies.

Types of cultures used to study the effect of AP infection by APMV on the virulence: Of the 41 studies examined in this systematic review, 35 studies identified the cultures utilised to study effect of AP infection by APMV on their virulence, which included five main types: *Acanthamoeba* cultures, co-culture systems, monolayer cell cultures, environmental and simulated cultures, and in vitro cultures with host modifications. Each culture type was employed in various studies to explore different aspects of infection dynamics, molecular mechanisms, cellular interactions, and ecological adaptations.

The virulence factors of APMV and AP affected by infection and evaluation of the impact on each factor: Published studies on the APMV infections of AP categorize the infection into two major types of infections, lytic and persistent infections (Mutsafi *et al,* 2010). APMV lytic infection of AP undergoes three stages, early, intermediate and late stages. During the early stage of lytic infection, several studies have reported a wide spread up-regulation of host genes that enhances the virulence of AP by supporting key activities related to the virulence and energy production (Mutsafi *et al,* 2013). These activities begin to decline during the intermediate and late stages of infection, eventually halting with the lysis of the host cell. Thus, expression of various virulence factors during symbiotic interactions is influenced differently, depending on various internal and external environmental aspects of symbiosis. The either interaction types between APMV and AP result in significant alterations in both the host and virus transcriptase's; indicating the various molecular events occurring during the infection process. These alterations generally affect DNA processes, alongside the regulation of the cytoskeleton and the equilibrium of intracellular oxidative levels. Nuri *et al,* (2022) reported that several cellular variations in AP during APMV infection that has notable effects on virulence of AP cells, and cellular variations were detected in host gene expression, membrane dynamics, cytoskeleton integrity and cell cycle progression.

Phagocytosis: APMV exhibits various characteristics that induce AP phagocytosis of the virus (Scola *et al,* 2003). Characteristics of APMV that enhance its phagocytosis by AP include the virus size, which is akin to bacterial size, as the virus diameter, including surface fibres was 0.7-.75µm (Zauberman *et al,* 2008). These surface fibres represent an unprecedented virus morphology enhanced AP phagocytosis of APMV (Klose *et al,* 2015). The fibres are composed mainly of four proteins: L725, L829, R135 & R856 (Sobhy *et al,* 2015). Fibres are covered with a peptidoglycan layer resembling Gram positive bacterial cell wall surface that aids in disguising virus as bacterial cells, thereby enhancing its attachment to amoebic cell re-

ceptors (Kuznetsov *et al,* 2010). AP phagocytic efficiency toward APMV is also affected by the organisms' diversity in both the external and internal environments. The effect of the symbiotic repertoire inside amoeba interferes with the phagocytic virulence of AP. Boyer *et al,* (2011) reported this effect compared AP phagocytosis efficiency of APMV in allopatric and bacterially sympatric conditions. The efficiency of APMV phagocytosis was higher in absence of bacteria. Phagocytosis in AP can be induced or hindered during various AMPV stages. Yaakov *et al,* (2019) by stochastic optical reconstruction microscopy found that super-resolution microscopy directly visualized infected AP cells, structures of tubulin and actin. They added fragmentation and polar accumulation of tubulin, as well as polarization and retraction of actin from the *Acanthopodia*. Alterations of microtubules and microfilaments caused rounding of infected cells, without *Acanthopodia* formation at both intermediate and final stages (about 4HPI) of infection, indicating the impairment of phagocytosis process. Silva *et al,* (2016) by PCR showed that mimivirus modulated expression of eight viral translation-related genes according to amoeba growth, with a higher induction of gene expression under starvation. But, some isolates differed translation-related gene expression; polymorphisms in promoter regions correlated with these differences. They added that mimivirus modulated the express- ion of translation-related genes responds to nutrient availability in the host cell, allowing them to adapt to different hosts growing under different nutritional conditions. Whether these alterations of the cellular cytoskeleton are transient and whether the cytoskeleton's regular structure might be restored in *A. castellanii* and cytoskeleton integrity might be restored later in the infection; however, this restoration could be species-dependent AP phagocytosis can also be affected by APMV infection in an indirect manner (Goyal *et al,* 2022). The production of these sugars was attributed to the R707 gene product of the virus. This gene encodes a putative glycosyltransferase enzyme that is a paralog to the human glycogenin-1 (GYG1) protein. This enzyme's glycosylation activity leads to the synthesis of virus-specific glycan chains that are incorporated into the virus's glycosylated fibres. Virus-specific surface fibres neither favoured by phagocytic glycan-binding receptors, but also mimic the Gram-positive bacterial cell wall (Rommel *et al,* 2016).

Encystment**:** One of the main virulence factors of AP is its ability to switch between trophozoite and cyst forms, with cyst formation providing a highly resistant state that enhances its survival and persistence under stress conditions. Trophozoite is the feeding stage of amoeba providing energy and metabolites necessary for virus replication, but cyst is a dormant stage that hinders viral replication. The encystment processes are influenced by various factors, including both external and internal environmental conditions, such as the APMV presence and other symbiotic organisms within AP (Anacarso *et al,* 2010). It was shown that APMV can prevent AP cyst formation by inhibiting expression of AP subtilizing-like encystation-mediating serine proteinase (EMSP) needed for encystation process. Gene silencing and fluorescence microscopy showed the expression and vesicle loading of enzyme and highlighted the EMSP crucial role in AP cyst formation (Moon *et al,* 2008). Boratto *et al,* (2015) reported that the virus can prevent AP cyst formation due to down-regulating EMSP expression led to a cell cycle arrest in most of the amoebae in a population subjected to APMV. They added that virus's ability to prevent the overall triggering of encystment process. Alternatively, APMV can prevent AP cyst formation during infection by action of various virally encoded proteins, such as Ras-related in brain guanosine diphosphatase (Rab GTPase), R387, L442, L724 and L829. The Rab GTPase enzyme, through its switching activity between GTP and GDP, can control several AP intracellular path-

ways that affect the movement, docking and fusion of vesicles, thereby preventing cyst formation in AP. Gene deletion studies of the APMV R387, L442, L724 and L829 proteins confirmed their roles in inhibiting AP encystation, but the mechanism was not elucidated (Ku *et al,* 2017). Also, APMV interferes with AP encystment by inducing cell cycle arrest, as reported by transcriptomic and experimentally (Nuri *et al,* 2022). Cytoskeletal homeostasis and structural integrity of infected AP are directly affected by alterations in gene expression in various infections, and genotoxic agents cause DNA damage, if not repaired causes chromosomal changes, and gene mutations, cancer formation or cell death (Christmann *et al,* 2003).

Environmental sensation and adaptive responses, including motility: In AP, environmental sensation and adaptive response are essential characteristics that significantly affect virulence (Suzan-Monti *et al,* 2007). AP environmental sensation depends on Filopodia and *Acanthopodia*; filopodia are responsible for detection, but Acanthopodia give driving force. Filopodia formation requires active polymerization and depolymerisation. This depends on actin, dynamin-II, & phosphoinositide 3-kinases or PI3Ks (Ghigo *et al,* 2008). The virus during AP infections showed distinct cytoskeletal rearrangement, including formation of viral factories occupying vast spaces in cytoplasm, enlargement of the nucleus, and aggregation of endoplasmic reticulum around viral factories, especially in infection late phase (Kuznetsov *et al,* 2010). Cytoskeleton re-arrangement is caused by drastic alterations in microtubule and actin microfilaments leading to impaired function of filopodia and acanthopodia, and irregular trafficking of actin following AP infection with APMV. Yaakov *et al,* (2019) observed impairment of the regular structure and function of filopodia during the intermediate and late phases of the lytic infection. AP cells infected with APMV *in-vitro* exhibited actin accumulation in cell poles after retraction from filopodia and acanthopodia resulting in the formation of shell-like structures in AP cells. The loss of environmental sensation and adaptive response to environmental stress renders infected cells more susceptible to infections and cellular damage (Kuznetsov *et al,* 2013). This was supported the tubulin cleavage phenomenon and actin filament network depolarization and cell cycle arrest that facilitates APMV replication and assembly (Goyal *et al,* 2022). Conversely, the up-regulation of genes associated with cytoskeleton regulation was reported during the early phase of lytic infection indicating enhancement of environmental sensation and adaptive response during early infection phase (Carty *et al,* 2021)

Gene transfer**:** The discovery of APMV marked a significant milestone in virology, as it was the first identified virus with a genome size exceeding one mega base, encoding about a 1000 predicted genes (Legendre *et al,* 2010). APMV genome contains genes typically found only in free-living organisms with those related to transcription and translation as transcription factors, tRNA, and amino-acyl-tRNA synthetases, along with genes involved in macromolecular metabolism and epigenetic regulation, such as protein modification, folding, and DNA repair (Raoult *et al,* 2004). Evidence suggests horizontal gene exchange between APMV and the AP genome, with gene transfer also reported between AP endosymbionts. The acquisition of these genes is an inherent characteristic of NCLDV viruses including the mimivirus. APMV has acquired numerous genes, including related to the ATP-dependent DNA ligase and various DNA repair enzymes are core elements of nucleocytoplasmic large DNA viruses (NCLDV), indicating a direct effect of gene transfer from other organisms (Filee *et al,* 2008). This genetic acquisition is facilitated by AP's role as a reservoir for diverse endosymbiosis, including other amoebophages, bacteriophages, virophages, bacteria, and protists increasing the potential for genetic transformation and recombination (Cohen *et al,* 2011). Gene tran-

sfer allows virus to acquire genes facilitating APMV independence from its host adapting to different environments by undertaking distinct metabolic activities from host (Simón *et al,* 2021). Moreira and Brochier-Armanet (200) found that four genes acquisition by APMV from AP: RAS GTPase (MIMI_ R214) & three serine/threonine protein kinases (MIMI_ R818, MIMI_R826 & MIMI_ R831). The virus utilizes the expression of these genes to alter host cell cycle. Homology of viral and amoebic proteins at 10% indicated horizontal transfer during organisms' coevolution (Chelkha *et al,* 2018). The amoeba serves as a gene melting pot, as it harbours many organisms from various biological types as endosymbionts and possesses the genes necessary for genome exchange between these organisms, including AP itself (Boyer *et al,* 2009). APMV has acquired 96 genes from bacteria, along with many bacterial-like mobile genetic elements (MGEs) such as insertion sequences, homing endonucleases, and several inteins. Filee *et al,* (2007) reported in their phylogenetic studies a restriction & modification system in which several MGEs were detected in synteny, and implying co-inheritance events. These transferred genes located to the ends of viral genome, occupying approximately 250 kb at each end (Filee *et al,* 2008).

Boyer *et al,* (2011) reported significant genome size reduction, resulting in altered APMV and AP morphology. The wild strain of APMV (M1) was recultivated in up to 150 aliquots under axenic conditions leading to the detection of a new strain of the virus that lacked the surrounding fibre of the capsid (M4 strain). The new strain differed from the wild strain in genome size that was reduced by 16%, with a loss of about 2.7 kbp. Sporadic deletions in various genes were detected but most deletions occurred within two large regions, 90.7 & 95.6 kbp, at both ends of genome encoded proteins didn't necessary under sterile conditions (Jegousse *et al,* 2017). Therefore, comparative proteomic analysis on M1 and M4 coupled with gel electrophoresis of the fibre in M1 performed in the study on M4 showed absence of two fibre proteins, L829 & R135. Genes loss of coding for L829 and R135 demonstrates selective disposal of specific genes under conditions where these phenotypic traits are not needed (Encarnación *et al,* 2005). Thus, the mimicking of bacterial cell wall is not required due to absence of competition with bacteria for binding with AP receptors that led the virus to eliminate these unnecessary genes, added to genome size reduction and fibre loss, other morphological alterations in AP occurred as changes in viral morphology factories, no peripheral electron-lucent zone where capsid acquires its fibres, and reduced lysis time enhancing viral virulence (Kim *et al,* 2023)

Symbiosis effects between AP, APMV, & endosymbionts of AP on infection dynamics causing changes in virulence: The ventual APMV infection outcome of AP cells is the lysis of amoebae within 12-24 hours post-infection or HPI (Suzan-Monti *et al,* 2006). However, APMV was shown to persist as an endosymbiont in AP for extended periods, reaching up to a year (Dornas *et al,* 2014). The symbiotic interaction within amoeba benefits the virus from a structural evolutionary perspective, as the viral genome has acquired genes from archaea, bacteria, and eukaryotes (Burki *et al,* 2021). Viral fibre structure is coated with a peptidoglycan layer resembling a Gram-positive bacterial cell wall (Raoult *et al,* 2007). The containment of viral genome within a supported envelope demonstrates similarity to eukaryotic cells. APMV exhibits bacteriophage-like features, including a unique structural 'stargate' at vertex as contractile tail of Myoviridae family of bacteriophages (Xiao *et al,* 2009). The virus can infect AP and survive for extended periods. Also, both can cause similar respiratory conditions, indicating stable co-evolution between the two organisms. APMV particles are stable and infectious in various environments, surviving for 9, 10, & 12 months in saltwater, ventilator devices, and fresh

water, respectively (Dornas *et al,* 2014). These particles exhibit high resistance to various biocidal agents, except for glutaraldehyde, which significantly affects viral titration reached 100% virucidal activity after 5 minutes at a 0.3% concentration (Campos *et al,* 2012). Within AP, virus survived for extended periods, resisting many environmental conditions, including those encountered during sewage treatment, and APMV stability was enhanced under extreme conditions, including heat, ultraviolet radiation, and exposure to various disinfectants, sterilisers, and antiseptics (Boratto *et al,* 2013).

Tripartite symbiotic system: A giant virus and virophages can interact within an amoebic cell in a tripartite symbiotic system. In this system AP acts as the host for two virus- es: APMV and a virophage co-infecting AP cells concurrently. APMV parasitizes AP ac-ting as a helper virus for virophage replication on the other, but virophage acts as a pa-rasite of the giant virus and a hyper-parasite of amoeba. Although the term 'virophage', coined by La Scola *et al,* (2008), implied the infection of a virus particle, the virophage in fact targets the viral factories of the giant virus within the host AP cell and utilises these factories for its replication rather than physically phagocytosing giant virus. These three virophages infected APMV using culture-based methods as Sputnik 1, Sputnik 2, and Sputnik 3, sharing 99% sequence identity (Gaia *et al,* 2013). Sputnik 1 was the first virophage to be identified and was considered a satellite virus due to its need for a helper giant virus to replicate and named after the 1st satellite, and successfully used (Fischer, 2021). Sputnik 1 was detected in a co-infection with an APMV new strain named *Acanthamoeba castellanii* mamavirus. This virus was larger than APMV, hence the name 'mamavirus', derived from root 'mama', meaning mother. The virophage also infects AP in co-infection with APMV. Co-infection of Sputnik 1 and APMV in AP yielded a lower viral load than infections with mamavirus alone, and Sputnik 2 was also discovered alongside another newly identified giant virus, 'lentillevirus', infecting the AP in a contact lens solution from a keratitis patient (Desnues and Raoult, 2010). The discovered giant virus was named lentillevirus, from the French word 'lentille,' referring to a lens, and the new virophage was named Sputnik 2 due to its high genome sequence similarity to Sputnik 1 (La Scola *et al,* 2010). Sputnik 3 was found in a soil sample associated with various mimiviruses from lineage C. Sputnik 1-3 virophages were all shown to infect AP in association with APMV but, Sputnik 3 infects AP independently of any giant virus (Gaia *et al,* 2013). Sputnik 1-3 utilise the viral factory for replication resulting in production of deformed APMV virions and a reduction in APMV replication, infectivity, and lytic capability (La Scola *et al,* 2008). Early studies indicated that virophages utilised viral fibres to enter amoebae (Desnues and Raoult, 2010). Boyer *et al,* (2011) reported that the virophage has a high affinity for the R135 protein, a surface fibre protein of APMV, which may promote the co-infection of AP cells by APMV and the virophage. PCR and immunofluorescence studies by the authors on AP cultures demonstrated successful propagation of the virophage in the M1 strain when co-infected with Sputnik 1 and Sputnik 2 and APMV, but not in the M4 strain, which lacks the surface fibres, confirming the utilisation of viral fibres by the virophage for cell entry.

Virophage infection generally diminishes APMV virulence by using the viral factories for its replication, thereby disrupting the normal life cycle of APMV in AP and increasing AP survival. Thus, Sputnik and AP can augment APMV virulence by facilitating gene transfer. Sputnik was shown to act as a gene transfer tool, aiding APMV in acquisition of various virulence factors. Also, AP contributes to this augmented gene transfer by serving as a melting pot for gene exchange (Gaia *et al,* 2013). A well-known example of gene transfer in this tripartite sym-

biotic interaction is the resistance of APMV to another virophage, named Zamilon (Boughalmi *et al,* 2012). Zamilon was shown to infect mimiviruses from lineages B and C, but cannot infect lineage A mimiviruses, including APMV (Gaia *et al,* 2014). Levasseur *et al,* (2016) reported that APMV has developed a nucleic acid-based immune mechanism against Zamilon, similar to clustered regularly interspaced short palindromic repeats (CRISPR)-Cas Type II-B system. The system was named mimivirus resistance element (MIMIVIRE) after they reported successful propagation of Zamilon in APMV by silencing the Zamilon insertions in APMV genome. The system includes three APMV genes: R349, R350, and R354. R349 contains two 28-nucleotide stretches inserted from Zamilon ORF4 gene, three 15-nucleotide repeats derived from Zamilon insertion, and four spacer sequences. R350 has helicase activity, and R354 required in the system for dual nuclease activity (Dou *et al,* 2018).

Conclusion

AP infection with AMPV affects various virulence factors including phagocytosis, cyst formation, environmental sensing, mobility, gene transfer and extended survival in symbiosis of organism including and this affect fluctuate depending on the infection stage. The effects can be species dependent. Environmental conditions and endosymbiosis have a significant effect on the effect of these virulence factors.

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Table 1: Summary of analysed studies and their types, culture and media used.

S. N.	Study	Study type	Culture	Media
1	Scola et al, (2003)	Cellular and Morphological Analysis	A. culture	PYG
2	Raoult et al, (2004)	Comparative Genomics	A. culture	PYG
3	Suzan-Monti et al, (2006)	Cellular and Morphological Analysis	A. culture	PYG
4	Filee et al, (2007)	Comparative Genomics	Not used	Not used
5	Suzan-Monti et al, (2007)	Cellular and Morphological Analysis	A. culture	PAS
6	Filee et al, (2008)	Comparative Genomics	Not used	Not used
7	Ghigo et al, (2008)	Phagocytosis and Host-Pathogen Interactions	Monolayer THP-1 and RAW 264.7 Vero cells monolayer	RPMI 1640, 10% FCS DMEM, 10% FCS
8	la Scola et al, (2008)	Symbiosis and Co-Evolution	Co-culture	NNA
9	Moon et al, (2008)	Phagocytosis and Host-Pathogen Interactions	A. culture	PYG
10	Moreira and Brochier-Armanet (2008)	Tripartite Symbiosis and Viral Ecology comparative genomics	Not used	Not used
11	Zauberman et al, (2008)	Cellular and Morphological Analysis	AP monolayer culture	DMEM and RPMI 1640, FCS
12	Boyer et al, (2009)	Tripartite Symbiosis and Viral Ecology	Co-culture	PYG
13	Xiao et al, (2009)	Environmental and Adaptation Studies	Not specified	NAS
14	Anacarso et al, (2010)	Phagocytosis and Host-Pathogen Interactions	Co-culture	PYG
15	Desnues and Raoult (2010)	Symbiosis and Co-Evolution	A. culture	PYG
16	Kuznetsov et al, (2010)	Cellular and Morphological Analysis	A. culture	Not specified
17	la Scola et al, (2010)	Symbiosis and Co-Evolution	Co-culture	PAS
18	Legendre et al, (2010)	Transcriptomic and Genomic Analyses	A. culture	PYG
19	Mutsafi et al, (2010)	Transcriptomic and Genomic Analyses	A. culture	PYG
20	Boyer et al, (2011)	Comparative Genomics Structural and Biochemical Studies	In vitro cultures with host modifications involved A. culture	NAS PYG
21	Cohen et al, (2011)	Tripartite Symbiosis and Viral Ecology	Co-culture	NAS
22	Boughalmi et al, (2012)	Symbiosis and Co-Evolution	Co-culture	PYG
23	Campos et al, (2012)	Environmental and Adaptation Studies	A. culture	PYG
24	Boratto et al, (2013)	Environmental and Adaptation Studies	A. Culture SEM	PYG
25	Gaia et al, (2013)	Symbiosis and Co-Evolution Environmental and Adaptation Studies	Co-culture A. culture	NNA PYG
26	Kuznetsov et al, (2013)	Molecular Characterisation and Functional Analysis	A. culture	PYG
27	Mutsafi et al, (2013)	Transcriptomic and Genomic Analyses	A. culture	PYG
28	Dornas et al, (2014)	Environmental and Adaptation Studies	A. culture	PYG
29	Gaia et al, (2014)	Symbiosis and Co-Evolution	Co-culture	PAS and PYG
30	Boratto et al, (2015)	Phagocytosis and Host-Pathogen Interactions	Co-culture	PAS
31	Klose et al, (2015)	Cellular and Morphological Analysis	A. culture	PBS
32	Sobhy et al, (2015)	Structural and Biochemical Studies	A. culture	PYG
33	Levasseur et al, (2016)	Comparative Genomics	Not used	Not used
34	Rommel et al, (2016)	phagocytosis and Host-Pathogen Interactions	A. culture E. coli BL21(DE3)	PYG
35	Ku et al, (2017)	Functional and In Vitro Studies	RIL strain	Not specified
36	Chelkha et al, (2018)	Tripartite Symbiosis and Viral Ecology	Not used	Not used
37	Dou et al, (2018)	Structural and Biochemical Studies	Not used	Not used
38	Yaakov et al, (2019)	Structural and Biochemical Studies	A. culture	PYG
39	Simón et al, (2021)	Comparative Genomics	Not used	Not used
40	Goyal et al, (2022)	Molecular Characterisation and Functional Analysis	A. culture	PYG
41	Nuri et al, (2022)	Molecular Characterisation and Functional Analysis	A. culture	PYG

Table 2: Newcastle-Ottawa Scale (NOS) assessment of the quality in systematic reviews.

Study	D1: Methodological quality Max: 4 stars					D2: Comparability of results Max: 2 stars			D3: Findings measures max: 3 stars				Adjusted overall	Overall /9
	D1a	D1	D1c	D1	Total	D2a	D2	Total	D3a	D3	D3c	Total		
Scola et al, (2003)	★	☆	★	★	★★★☆	★	★	★★	★	★	★	★★★	8/8	9
Raoult et al, (2004)	★	☆	☆	★	★★☆☆	★	★	★★	★	☆	★	★★☆	7/7	9
Suzan-Monti et al, (2006)	★	☆	★	★	★★★☆	★	★	★★	★	☆		★☆	6/7	8
Filee et al, (2007)	☆	☆	☆	★	★☆☆☆	★	★	★★	★	☆	★	★★☆	5/5	9
Suzan-Monti et al, (2007)	★	☆	★	★	★★★☆	★	★	★★	★	☆	★	★★☆	7/7	9
Filee et al, (2008)	★	☆	★	★	★★★☆	★	★	★★	★	☆		★☆	6/7	8
Ghigo et al, (2008)	★	☆	★	★	★★★☆	★	★	★★	★	★	★	★★★	8/8	9
la Scola et al, (2008)	★	☆	★	★	★★★☆	★	★	★★	★	★	★	★★★	8/8	9
Moon et al, (2008)	☆	☆	☆	★	★☆☆☆	★	★	★★	★	☆	★	★★☆	5/5	9
Moreira et al, (2008)	☆	☆	☆	★	★☆☆☆	★	★	★★	★	☆	★	★★☆	5/5	9
Zauberman et al, (2008)	★	☆	★	★	★★★☆	★	★	★★	★	☆	★	★★☆	7/7	9
Boyer et al, (2009)	★	☆	★	★	★★★☆	★	★	★★	★	☆	★	★★☆	7/7	9
Xiao et al, (2009)	★	☆	★	★	★★★☆	★	★	★★	★	☆	★	★★☆	7/7	9
Anacarso et al, (2010)	★	☆	★	★	★★★☆	★	★	★★	★	☆	★	★★☆	7/7	9
Desnues and Raoult (2010)	★	☆	★	★	★★★☆	★	★	★★	★	☆	★	★★☆	8/8	9
Kuznetsov et al, (2010)	★	☆	★	★	★★★☆	★	★	★★	★	☆	★	★★☆	7/7	9
la Scola et al, (2010)	★	☆	★	★	★★★☆	★	★	★★	★	☆	★	★★☆	7/7	9
Legendre et al, (2010)	★	☆	★	★	★★★☆	★	★	★★	★	★	★	★★★	8/8	9
Mutsafi et al, (2010)	★	★	★	★	★★★★	★	★	★★	★	★	☆	★★★	8/8	9
Boyer et al, (2011)	★	★	★	★	★★★★	★	★	★★	★	★	★	★★★	9/9	9
Cohen et al, (2011)	★	☆	★	★	★★★☆	★	★	★★	★		★	★★	7/8	8
Boughalmi et al, (2012)	★	☆	★	★	★★★☆	★	★	★★	★	☆	★	★★☆	7/7	9
Campos et al, (2012)	★	★	★	★	★★★★	★	★	★★	★	★		★★	8/9	8
Boratto et al, (2013)	★	★	★	★	★★★★	★	★	★★	★	★	★	★★★	9/9	9
Gaia et al, (2013)	★	★	★	★	★★★★	★	★	★★	★	★	★	★★★	9/9	9
Kuznetsov et al, (2013)	★	★	★	★	★★★★	★	★	★★	★	★	★	★★★	9/9	9
Mutsafi et al, (2013)	★	☆	★	★	★★★☆	★	★	★★	★	★	★	★★★	8/8	9
Dornas et al, (2014)	★	☆	★	★	★★★☆	★	★	★★	★	★	★	★★★	8/8	9
Gaia et al, (2014)	★	★	★	★	★★★★	★	★	★★	★	★	★	★★★	9/9	9
Boratto et al, (2015)	★	☆	★	★	★★★☆	★	★	★★	★	★	★	★★★	8/8	9
Klose et al, (2015)	★	★	★	★	★★★★	★	★	★★	★	★	★	★★★	9/9	9
Sobhy et al, (2015)	★	★	★	★	★★★★	★	★	★★	★	★	★	★★★	9/9	9
Levasseur et al, (2016)	★	★	★	★	★★★★	★	★	★★	★	★	★	★★★	9/9	9
Rommel et al, (2016)	★	☆	★	★	★★★☆	★	★	★★	★	★	★	★★★	8/8	9
Ku et al, (2017)	★	☆	★	★	★★★☆	★	★	★★	★	☆	★	★★☆	7/7	9
Chelkha et al, (2018)	☆	☆	☆	★	★☆☆☆	★	★	★★	★	☆	★	★★☆	5/5	9
Dou et al, (2018)	★	☆	☆	★	★★☆☆	★	★	★★	★	☆	★	★★☆	6/6	9
Yaakov et al, (2019)	★	★	★	★	★★★★	★	★	★★	★	★	★	★★★	9/9	9
Simón et al, (2021)	☆	☆	☆	★	★☆☆☆	★	★	★★	★	☆	★	★★☆	5/5	9
Goyal et al, (2022)	★	☆	★	★	★★★☆	★	★	★★	★	★	★	★★★	8/8	9

★: Star is awarded for the study that fulfils the subdomain of the NOS criteria, : No star is awarded for the study that does not fulfil the subdomain of the NOS criteria, ☆: Hollow star is awarded for non-applicable subdomain in the study. **D1a**: Representativeness of the exposed cohort, **D1b**: Selection of non-exposed cohort, **D1c**: Ascertainment of Exposure, **D1d**: Outcome is not present at start, **D2a**: Breadth of techniques, **D2b**: Control for additional factors, **D3a**: Quality of outcome assessment, **D3b**: Effect of a Virulence Factor on an Organism is assessed directly, **D3c**: Relevance of follow-up.