Mimiviridae, Marseilleviridae, and virophages as emerging human pathogens causing healthcare-associated infections

Mimiviridae, Marseilleviridae und Virophagen als Erreger von Healthcare-assoziierten Infektionen mit wachsender Bedeutung

Abstract

Aim: During the last decade it became obvious that viruses belonging to Mimiviridae and Marseilleviridae families (order Megavirales), may be potential causative agents of pneumonia. Thus, we have performed a review of the association of Mimiviridae, Marseilleviridae, and virophages with pneumonia, particularly healthcare-associated pneumonia, and other infections of the respiratory tract.

Results and discussion: According to the analysis of the published articles, viruses belonging to Mimiviridae family can be potential agents of both community-acquired and healthcare-associated pneumonia. In particular, these viruses may be associated with poor outcome in patients of intensive care units.

The exact mechanism of their pathogenicity, however, still remains unclear. The discrepancies between the results obtained by serological and genomic methods could be explained by the high polymorphism of nucleotide sequences of Mimiviridae family representatives. Further investigations on the Mimiviridae pathogenicity and on the determination of Mimiviridae-caused pneumonia risk groups are required.

However, the pathogenicity of the viruses belonging to Marseilleviridae family and virophages is unclear up to now.

Keywords: mimivirus, mimiviridae, marseilleviridae, giant viruses, virophages, pneumonia, healthcare-associated infections

Zusammenfassung

Zielsetzung: Im letzten Jahrzehnt wurde vermutet, dass Viren der Familie der Mimiviridae und der Marseilleviridae (sog. Megavirales) Pneumonien verursachen können. Deshalb wurde eine Literaturrecherche zu den möglichen Zusammenhängen von Mimiviridae, Marseilleviridae, Virophagen und Pneumonien mit den Schwerpunkten HA-Infektionen und andere Infektionen der Atemwege durchgeführt.

Ergebnisse und Diskussion: Die Analyse ergab, dass Viren aus der Familie der Mimiviridae potentielle Verursacher sowohl der CA- als auch der HA-Pneumonie sein können. Diese Viren können zu einem verschlechternen Outcome bei Intensivtherapiepatienten führen. Der genaue Mechanismus ihrer Pathogenität ist jedoch noch immer nicht geklärt. Die unterschiedlichen Ergebnisse zwischen serologischen und Genommethoden sind wahrscheinlich durch den hohen Polymorphismus der Nucleotidsequenzen der Vertreter der Mimiviridae zu erklären. Daher sind weitere Untersuchungen zur Pathogenität der Mimiviridae und ihrer Rolle bei der Pneumonieentstehung in Risikogruppen notwendig.

Im Unterschied dazu ist die Pathogenität der Viren der Familie der Marseilleviridae und der Gruppe der Virophagen noch unklar.

Anton G. Kutikhin1,2,3
Arseniy E. Yuzhalin*4
Elena B. Brusina1,3

1 Department of Epidemiology, Kemerovo State Medical Academy, Kemerovo, Russian Federation
2 Central Research Laboratory, Kemerovo State Medical Academy, Kemerovo, Russian Federation
3 Research Institute for Complex Issues of Cardiovascular Diseases under the Siberian Branch of the Russian Academy of Medical Sciences, Kemerovo, Russian Federation
4 Department of Oncology, Cancer Research UK and Medical Research Council Oxford Institute for Radiation Oncology, University of Oxford, Oxford, United Kingdom
**Introduction**

The existence of viruses with extremely large particle and genome sizes has been predicted since the discovery of jumbo bacteriophages in the 1970s and the **phycodnaviruses** in the early 1980s [1], [2]. An investigation of pneumonia outbreak with no clear causative agent in Bradford, England (1992) by Tim Rowbotham and his team considered water of a cooling tower as a probable origin of the outbreak and amoebae as potential culprits [3] since they were established as Trojan horses due to their ability to harbor multiple agents of human pneumonia that can survive and multiply under the protection from various external physical and chemical agents within the amoebae encyst [4], [5]. This led to an isolation of an amoebal pathogenic bacteria, one of which had the appearance of a Gram-positive coccus and was therefore named Bradford coccus [3], [6], [7], [8], [9]. However, it resisted the PCR amplification and 16S ribosomal DNA sequencing. This led to the observation of Bradford coccus using electron microscopy, which revealed that it had a viral icosahedral structure. The viral nature of Bradford coccus was further confirmed by an isolation phase during its replication cycle [6], and genome sequencing [10]. In 2003, the Bradford coccus was renamed as *Acanthamoeba polyphaga Mimivirus* (APMV) due to its mimicry of a bacterium by its size and appearance by Gram staining [3]. Raoult et al. [3], [10] reported that APMV genome was the largest among all of viruses (1,181 kb), encoding more than 900 proteins, including those never identified previously in viruses. Overall, the **Mimivirus** discovery has resulted in a considerable shift in our understanding of the definition, origin, and evolution of viruses [11], [12]. **Mimiviruses** were classified into a separate group of viruses called **nucleocytoplasmic large DNA viruses** (NCLDVs) which was first described in 2001 [13] and included the families of **Poxviridae**, **Asfarviridae**, **Iridoviridae**, **Ascorviridae**, and **Phycodnaviridae** [14], [15], [16]. In 2007, a first member of the **Marseilleviridae** family related to NCLDVs, *Acanthamoeba polyphaga marseillevirus* (APMaV), was isolated from water collected from a cooling tower in Paris, France, using a method based on *Acanthamoeba polyphaga* culture [17]. This virus was named in honor of its amoebal host and of the name of the French city, Marseille, where it was discovered [17]. The Marseillevirus was characterized by a 368-kb genome, 457 genes, and a minimum of 49 proteins [17]. Furthermore, the first member of the second branch of **Mimiviridae** family, *Cafeteria roenbergensis* virus (CrOv), was described in 2010 as an agent infecting marine zooplankton, and its genome consisted of $\approx 730$ kb of double-stranded DNA [18]. Recently, Colson et al. [19], [20] proposed assigning an official taxonomic rank to the NCLDVs as the order **Megavirales**, due to the large size of the virions and genomes of these viruses, and because of their common ancestral origin [13], [15], [21], [22]. Families and genus belonging to this new tentative order are summarized in Table 1, whilst a brief timeline of discoveries in this field is presented in Table 2. During the last decade, plenty of other giant virus strains belonging to **Mimiviridae** and **Marseilleviridae** families were discovered [23], and their diversity is reflected in Table 3 and Table 4.

For decades, viruses were considered to be a final level of parasitism in living nature. However, in 2008, La Scola et al. [24] described an icosahedral small virus Sputnik (50 nm in size) which was associated with an APMV. Sputnik did not replicate in *Acanthamoeba castellanii* but demonstrated a rapid growth in the giant virus factory revealed in amoebae co-infected with APMV. The most incredible fact was that Sputnik life cycle was harmful for APMV and led to the production of abortive forms and abnormal capsid assembly of the host virus. The authors suggested that Sputnik belongs to a new family of viruses and classified it as a **virophage** [24]. In 2011, Fischer and Suttle [25] described a new virophage called **Mavirus**, and Yau et al. [26] almost simultaneously reported a discovery of **Organic Lake Virophage**. Eighteen months later, Desnues et al. [27] revealed a new Sputnik strain, which had 99% identity to the original Sputnik virophage genome sequence. A recent comprehensive investigation carried out by Zhou et al. [6] suggested an existence of five new virophages, namely Yellowstone Lake Virophages 1, 2, 3, 4, and **Ace Lake Mavirus**. Finally, third Sputnik strain was described in 2013 by Gaia et al. [7]. The discovery of virophages revolutionized our understanding of host-parasite interrelations. Features of known virophages are presented in Table 5.

There is a significant lack of knowledge regarding the biology of virophages. Obviously, they have greater importance in living nature then we can suggest at the moment. Virophages perform a control of non-viral host-virus dynamics as the essential regulators of ecological interrelations [26], participate in a transfer of genetic information between various organisms as the mobile genetic elements [25], particularly in an interviral gene transfer [27], may be the pathogens of the multicellular organisms [28], and can possibly be used as a new way to fight with the emerging viral infections. Bacteriophages are being successfully used against bacteria in a clinical practice; presumably, a similar pattern does not seem to be impossible in the case with virophages and human pathogenic viruses. Further research on the phenomenon of virophages will definitely shed light on their role in living nature. It was suspected that **mimiviruses** may be potential causative agents of pneumonia due to the setting of its initial discovery and to the involvement of some water-associated amoebae-resistant bacteria, including **Legionella pneumophila**, in such infections [3], [4]. Experimentally, **Mimivirus** was found to be capable of inducing...
pneumonia in mice [29] and infecting macrophages through phagocytosis [30]. A number of case reports showing the pathogenicity of viruses belonging to Mimiviridae and Marseilleviridae families in humans during the last decade (Table 6) [31], [32], [33], [28], [34], [35], [36], [37], [38]). Further, certain reports demonstrated that asymptomatic Marseillevirus infection is not rare in healthy persons and may be transmitted by transfusion [39]. In addition, the results of previous studies identified sequences related to members of families of the order Megavirales in human blood, nasopharyngeal samples, or stools [40], [41], [42], [43], [44], [45], [46]. Thus, we aimed to perform a review of the association of Mimiviridae, Marseilleviridae, and virophages with pneumonia, particularly healthcare-associated pneumonia (HAP), and other infections of the respiratory tract.

| Family            | Subfamily       | Genus            | Host range                    |
|-------------------|-----------------|------------------|-------------------------------|
| Ascoviridae       |                 | Ascovirus        | Insects                       |
| Asfarviridae      |                 | Asfivirus        | Mammals, dinoflagellates      |
| Iridoviridae      |                 | Chloriridovirus  | Insects                       |
|                   |                 | Iridovirus       | Insects                       |
|                   |                 | Lymphocystivirus | Fishes                        |
|                   |                 | Megalocytivirus  | Fishes                        |
|                   |                 | Ranavirus        | Amphibia                      |
| Mimiviridae       |                 | Mimivirus        | Amoeba                        |
|                   |                 | Cafeteria roenbergenensis virus (CroV) | Amoeba, green algae, heterokonts, haptophyta |
| Marseilleviridae  |                 | Marseillevirus   | Amoeba                        |
| Phycodnaviridae   |                 | Chlorovirus      | Green algae                   |
|                   |                 | Coccolithovirus  | Haptophyta                    |
|                   |                 | Phaeovirus       | Heterokonts                   |
|                   |                 | Prasinovirus     | Green algae                   |
|                   |                 | Raphidovirus     | Heterokonts                   |
| Poxviridae        | Chordopoxvirinae| Avipoxvirus      | Birds                         |
|                   |                 | Capripoxvirus    | Mammals                       |
|                   |                 | Cervidpoxvirus   | Mammals                       |
|                   |                 | Crocodylifoxvirus| Reptiles                      |
|                   |                 | Leporipoxvirus   | Mammals                       |
|                   |                 | Molluscipoxvirus | Human                         |
|                   |                 | Orthopoxvirus    | Mammals                       |
|                   |                 | Parapoxvirus     | Mammals                       |
|                   |                 | Suipoxvirus      | Mammals                       |
|                   |                 | Yatapoxvirus     | Primates                      |
|                   |                 | Unassigned       | Animals                       |

### Table 1: Members of the proposed order Megavirales

| Family            | Subfamily       | Genus            | Host range                    |
|-------------------|-----------------|------------------|-------------------------------|
|                   |                 | Alphacentromopoxvirus | Insects                |
|                   |                 | Betaentomopoxvirus | Insects                       |
|                   |                 | Gammaentomopoxvirus | Insects                       |
|                   |                 | Unassigned       | Insects                       |

**Materials and methods**

To the best of our knowledge, all relevant articles published before December of 2013 and available in PubMed database were included in this review. The generation of search queries was performed by combination of words placing at certain positions in the structure of the query, and all feasible variants were browsed: *First position*: “mimivirus”, “mimiviruses”, “mimiviridae”, “marseillevirus”, “marseilleviruses”, “marseilleviridae”, “giant virus”, “giant viruses”, “nucleocytoplasmic large DNA virus”, “nucleocytoplasmic large DNA viruses”, or “megavirales”. *Second position*: “pneumonia”, “nosocomial”, “community-acquired”, “hospital-acquired”, “ventilator-associated”, “healthcare-associated”, or “respiratory”. Reference lists of all relevant articles were also screened for the papers which could elude from our search. According to the results of the search, we identified 11 relevant
Table 2: A brief timeline of discoveries regarding the nucleocytoplasmic large DNA viruses (NCLDVs) superfamily and the Megavirales order

| Year   | Discovery                                                                 |
|--------|---------------------------------------------------------------------------|
| 2001   | Comparative analysis of viral genomes with identification of a set of conserved genes led to the isolation of nucleocytoplasmic large DNA viruses (NCLDVs) into the separate group consisted of four families of viruses (Poxviridae, Asfarviridae, Indoviridae, and Phycodnaviridae) which had a double-stranded DNA genome greater than 200,000 bp in size and sharing 9 genes. |
| 2003    | Identification of the genome of Mimivirus, the largest virus known to the moment (1,181,404 bp), the definition of a new family among NCLDVs: Mimiviridae. |
| 2004    | Identification of Sputnik, a member of a new family of viruses that grew in the giant virus factory found in amoebae co-infected with Mamavirus, a new Mimivirus strain. This virus was considered as a virophage since it presented functional analogy with bacteriophages; it replicated only in the presence of Mamavirus and its growth was deleterious to the giant virus. The Sputnik genome consisted of a 18.343-kilobase circular double-stranded DNA. |
| 2008    | Isolation of Marseillevirus using amoebal culture of water collected in a cooling tower. Its genome represented a circular, double-stranded DNA molecule of 388,453 bp. It was shown by the phylogenetic analysis that Marseillevirus belongs to a new viral family of NCLDVs, Marseilleviridae. |
| 2009    | The NCLDVs core genes appear to have various probable origins including eukaryotes, bacteria, and bacteriophages. The results suggested that the NCLDVs originated at an early stage in the evolution of eukaryotes. |
| 2010    | Phylogeny reconstruction of highly conserved proteins revealed three main lineages named A (that includes Mimivirus), B (whose leading member is Moumouivirus), and C composed of several members including Courdo 11 and Tera1. |
| 2010    | The genome of the Cafeteria roenbergensis virus (CroV) was described, with an estimated size of 730 kbp. This virus infects a widespread marine heterotrophic flagellate. Phylogenetic reconstructions indicated that CroV is related to the Mimiviridae family, apart from the group composed by three lineages. |
| 2011    | The genome of Lausannevirus, a close relative of the Marseillevirus, was described in 2011. It was recovered using amoebal co-culture from a water sample collected in the Seine river that runs through Paris, France, had 346-kbp genome, and shared 89% of genes with Marseillevirus. |
| 2012    | The suggestion to consider Megavirales as a separate order consisting of seven families (Ascoviridae, Asfarviridae, Indoviridae, Mimiviridae, Marseilleviridae, Phycodnaviridae, Poxviridae). |

Results and discussion

Unfortunately, it was not possible to carry out a meta-analysis since due to significant differences in study design, sample types, and methods of detection used in distinct studies. Thus, we performed only the qualitative comparative analysis. The results of the first investigation on the association of APMV with pneumonia were published in 2005 by La Scola et al. [47]. The researchers collected serum samples from 376 Canadian patients with community-acquired pneumonia (CAP, 121 ambulatory and 255 hospitalized) and 511 healthy control subjects. Microimmunofluorescence assay revealed that patients with CAP were mimivirus-positive significantly more frequently compared to controls (9.66% vs. 2.30%, P<0.01) (Table 7). Furthermore, the authors identified hospitalization from a nursing home and rehospitalization after discharge as independent risk factors of mimivirus-associated CAP (P<0.05), possibly due to poor efficacy of standard antimicrobial agents against viruses. In addition, immunoelectron microscopy revealed that antibodies of APMV-positive patients specifically recognized mature APMV particles whilst antibody fixation was not found in serum samples from APMV-negative patients. The authors suggested APMV as a particularly hazardous etiologic agent of pneumonia acquired in institutions [47], [48]. In addition, the investigators recruited a second study sample consisted of 26 French patients with intensive care unit (ICU)-acquired pneumonia and 50 healthy controls, showing that APMV can be found in patients with ICU-acquired pneumonia and multiple populations [47]. Moreover, serological positivity to APMV was associated with a higher risk of ICU-acquired pneumonia (19.2% in cases vs. 0.0% in controls, P<0.01) [47] (Table 7). In the third sample (32 French patients with ICU-acquired pneumonia, 21 intubated controls in ICU without ICU-acquired pneumonia), APMV DNA was detected in bronchoalveolar lavage specimen from one of the patients with ICU-acquired pneumonia but in none of the controls, confirming that APMV may reach the respiratory tract of these patients [47] (Table 7). So, La Scola et al. [47] were very first who proposed that APMV should be tested as a possible novel human pathogen, particularly in pneumonia patients.

The second study devoted to this issue was carried out by Berger et al. [49]. This investigation included 157 French ICU patients with 210 episodes of pneumonia (120 episodes of healthcare-associated pneumonia (HAP), 62 episodes of CAP, and 28 episodes of mixed pneumo-
Table 3: Known members of the Mimiviridae family

| Group of giant viruses | Virus                                      | Source of identification          | Year, country and region of identification |
|------------------------|--------------------------------------------|-----------------------------------|-------------------------------------------|
|                        | Mimiviridae (44)                           |                                   |                                           |
| Group I (43)           |                                            |                                   |                                           |
| Group A (14)           | *Acanthamoeba polyphaga Mimivirus* (APMV)  | Cooling tower water              | 2003 [6], 2004 U.K. (Bradford) [10]       |
|                        | *Acanthamoeba castellanii Mamavirus*       | Cooling tower water              | 2008, France (Paris) [64]                 |
|                        | *Terra2*                                   | Soil                             | 2010, France (Marseille) [57]             |
|                        | *Pointe-Rouge2*                            | Seawater                         |                                           |
|                        | *Cher*                                     | Rivers and lakes                 | 2010, France (Tours) [57]                 |
|                        | *Fauteuil*                                 | Hospital water                   | 2010, France (Marseille) [57]             |
|                        | *Longchamps*                               | Decorative fountain water        | 2010, France (Marseille) [57]             |
|                        | *Lactours*                                 | Rivers and lakes                 | 2010, France (Tours) [57]                 |
|                        | *Pointe-Rouge1*                            | Seawater                         | 2010, France (Marseille) [57]             |
|                        | *Lentille*                                 | Lens liquid                      | 2010, France (Marseille) [57]             |
|                        | *Marais*                                   | Swamp                            | 2013, France (Aubagne) [23]               |
|                        | *Univirus*                                 | Compost                          | 2013, France (Marseille) [23]             |
|                        | *Hirudovirus*                              | Leech                            | 2013, France (Marseille) [23]             |
|                        | *Montadette2*                              | Soil                             | 2013, France (Martigues) [23]             |
| Group B (6)            | *Mou mou virus*                            | Cooling tower water              | 2010, France (Rousset) [57]               |
|                        | *Monve*                                    | Cooling tower water              | 2010, France (Puget sur Argens) [57]      |
|                        | *Ochan*                                    | Compost                          | 2013, France (Marseille) [23]             |
|                        | *Goulette*                                 | Seawater                         | 2000, Tunisia (Tunis) [64]                |
|                        | *Istres*                                   | Soil                             | 2013, France (Istres) [23]                |
|                        | *Cassis49*                                 | Soil                             | 2013, France (Cassis) [23]                |
| Group C (25)           | *Cou dro7*                                 | Rivers and lakes                 | 2010, France (Saint-Raphael) [57]         |
|                        | *Terra1*                                   | Soil                             | 2010, France (Marseille) [57]             |
|                        | *Montpellier*                              | Decorative fountain water        | 2010, France (Montpellier) [57]           |
|                        | *Cou dro11*                                | Rivers and lakes                 | 2010, France (Saint-Raphael) [57]         |
|                        | *Cou dro6*                                 | Rivers and lakes                 | 2010, France (Marseille) [57]             |
|                        | *Bus*                                      | Cooling tower water              | 2010, France (Marseille) [57]             |
|                        | *Mont1*                                    | Soil (mountain)                  | 2000, Tunisia (Tunis) [64]                |
|                        | *LBA111*                                   | Bronchoalveolar lavage           | 2013, Tunisia [65]                        |
|                        | *Avenue9*                                  | Soil                             | 2000, Tunisia (Tunis) [64]                |
|                        | *Afrovirus*                                | Soil                             | 2013, France (Aubagne) [23]               |
|                        | *Montadette1*                              | Soil                             | 2013, France (Martigues) [23]             |
|                        | *Balcon*                                   | Soil                             | 2013, France (Marseille) [23]             |
|                        | *Terrain en construction*                  | Soil                             | 2013, France (Marseille) [23]             |
|                        | *Boug1*                                    | Chott (hypersaline soil)         | 2000, Tunisia (Gafsa) [64]                |
|                        | *Shan*                                     | Stool                            | 2013, Tunisia (Tunis) [23]                |
|                        | *Cornil*                                   | Soil                             | 2013, France (Marseille) [23]             |
|                        | *Saint Pierre*                             | Stagnant water                   | 2013, France (Marseille) [23]             |
|                        | *Borely*                                   | Stagnant water                   | 2013, France (Marseille) [23]             |
|                        | *Capucin*                                  | Stagnant water                   | 2013, France (Marseille) [23]             |
|                        | *Potager*                                  | Soil                             | 2013, France (Marseille) [23]             |
|                        | *Feuillage*                                | Soil                             | 2013, France (Martigues) [23]             |
|                        | *Luminy43*                                 | Water                            | 2013, France (Marseille) [23]             |
|                        | *Sete*                                     | Soil                             | 2013, France (Sete) [23]                 |
| Group II (1)           |                                            |                                   |                                           |
| Unassigned             | *Cafeteria roenbergensis virus* (CroV)     | Unicellular marine biflagellate   | 2010, Coastal waters near the USA (Yaquina Bay, Oregon) [18] |
Acanthamoeba polyphaga
Marseillevirus (APMv)

Acanthamoeba castellanii
Lausannevirus (ACLAv)

Giant blood Marseillevirus

Senegalvirus

Table 4: Known members of the Marseilleviridae family

| Group of giant viruses | Virus | Source of identification | Year, country and region of identification |
|------------------------|-------|--------------------------|------------------------------------------|
| Marseilleviridae (17)  |       |                          |                                          |
| Marseillevirus (4)     | Acanthamoeba polyphaga Marseillevirus (APMv) | Cooling tower water | 2009, France (Cannes) [17] |
|                        | Acanthamoeba castellanii Lausannevirus (ACLAv) | River water | 2011, France [63] |
|                        | Giant blood Marseillevirus | Donor blood | 2013, France [66] |
|                        | Senegalvirus | Stool | 2010, Senegal [18] |
| Tunisivirus (1)        | Fontaine2 | Fountain water | 2000, Tunisia (Ariana) [64] |
| Unassigned (14)        | Cannes8   | Cooling tower water | 2010, France (Cannes) [57] |
|                        | Cannes9   | Cooling tower water | 2010, France (Cannes) [57] |
|                        | Saint-Charles | Decorative fountain water | 2010, France (Marseille) [57] |
|                        | Seb1 eau  | Sebkha (hypersaline water) | 2000, Tunisia (Tunis) [64] |
|                        | Seb1 sol  | Soil (hypersaline soil) | 2000, Tunisia (Tunis) [64] |
|                        | Seb6 sol  | Soil (hypersaline soil) | 2000, Tunisia (Tunis) [64] |
|                        | Seb2 sol  | Soil (hypersaline soil) | 2000, Tunisia (Tunis) [64] |
|                        | Oued1     | River | 2000, Tunisia (Bezert) [64] |
|                        | Cite1     | Soil | 2000, Tunisia (Kef) [64] |
|                        | Riviere1  | River (Majerda) | 2000, Tunisia (Kef) [64] |
|                        | Puit1     | Well water | 2000, Tunisia (Cap Bon) [64] |
|                        | Hammam1  | Hammam water | 2000, Tunisia (Tunis) [64] |
|                        | Sidi thabet | Soil | 2000, Tunisia (Ariana) [64] |
|                        | Insectomime | Diptere larvae | 2013, Tunisia [31] |

nia (CAP complicated with HAP) [49]. Using the similar microimmunofluorescence approach, the authors showed that 15/210 episodes (7.1%) in 14/157 patients (8.9%) were associated with APMV [49]. In addition, laboratory investigations for amoeba-associated microorganisms (AAMs) revealed 59/210 (28.1%) diagnoses in 40/157 (19.0%) patients [49]. Seroconversion among patients with ventilator-associated pneumonia (VAP) was observed in 13/51 episodes (25.5%), whereas seroconversion among patients with CAP was detected in 2/8 episodes (25.0%) that may demonstrate the possible pathogenic role of APMV in both VAP and CAP [49] (Table 7). However, there was no control group in this study; therefore the prevalence of APMV in a general population remains unknown [49]. In the same year, Arden et al. [50] and Larcher et al. [51] failed to find any APMV-positive individuals among 315 Australian patients with suspected acute respiratory tract infections and 214 Austrian patients with pediatric CAP, respectively; nonetheless, the authors used predominantly nasopharyngeal aspirates as a study material and real-time [50] or nested suicide [51] PCR instead of microimmunofluorescence as a method of APMV detection (Table 7). Similar results were further obtained by Dare et al. [52] who were unable to detect APMV using real-time PCR in 496 patients with pneumonia (249 patients with CAP, 71 patient with HAP, 87 bone marrow transplant recipients with pneumonia, and 89 lung transplant recipients with pneumonia) from 9 epidemiologically varied pneumonia patient populations (Thailand, Canada, USA) (Table 7). The authors suggested that seropositivity may reflect chronic exposure to APMV antigen rather than active infection, and the potential for nonspecific cross-reactions with the serologic assays used may have inflated the true prevalence of APMV colonization/infection [52]. Nevertheless, most of the specimens examined in this study were obtained from the upper respiratory tract but not from the lower respiratory tract where the only reported APMV PCR-positive sample was identified [47]. Distinct composition of the studies’ populations could also play a role in the differences between the studies [52]. However, in 2009 Vincent et al. [53] found 59/300 (19.6%) French patients with suspected VAP to be APMV-positive, detected by microimmunofluorescence (Table 7). In addition, APMV-positive patients had longer duration of mechanical ventilation and ICU stay with median excesses of 7 days and 10 days, respectively, so a positive serology for mimivirus was associated with a poorer outcome in mechanically ventilated ICU patients [53].
Table 5: Features of known virophages

| Year and region of the discovery | Cellular organism infected by the virus | Virus infected by the virophage | Virophage | Environment |
|----------------------------------|----------------------------------------|---------------------------------|-----------|-------------|
| 2008, Paris, France [24]         | *Acanthamoeba polyphaga* and possibly other protozoa, particularly amoebae | Mimiviruses from all 3 groups (A, B, and C) | Sputnik virophage | A cooling tower |
| 2011, Texas, USA [25]           | *Cafeteria roenbergensis* (marine phagotrophic flagellate) | *Cafeteria roenbergensis* virus (CroV), a distant relative of mimivirus (the same family of nucleocytoplasmic large DNA viruses (NCDLVs)) | Mavir | Coastal waters |
| 2011, Antarctica [26]           | Prasinophytes (photoautotrophic algae) | Phycodina- or mimivirus? | Organic Lake Virophage | Organic Lake, a hypersaline meromictic lake, temperature at the surface of the lake can vary from -14 to +15 °C while remaining subzero at depth [67] |
| 2012, France [27]               | *Acanthamoeba polyphaga* and possibly other protozoa, particularly amoebae | Mimiviruses from all 3 groups (A, B, and C) | Sputnik virophage 2 | Contact lens fluid of a patient with keratitis [32] |
| 2013, Wyoming, USA [36]         | Microalgae? | Phycodina- or mimiviruses? | Yellowstone Lake Virophages (1–4) | Yellowstone Lake, a freshwater lake with a temperature ranging from 12 to 73°C in Yellowstone National Park [69] |
| 2013, Antarctica [68]           | Phagotrophic protozoan? | Mimiviruses? | Ace Lake Mavirus | Ace Lake, a hypersaline meromictic lake, covered with ice for as long as 11 months to an entire year, with an average temperature of approximately 0°C [70] |
| 2013, South of France [65]      | *Acanthamoeba polyphaga* and possibly other protozoa, particularly amoebae | Mimiviruses from all 3 groups (A, B, and C) | Sputnik virophage 3 | Soil samples collected in Marseille and surrounding areas |

109 Dutch patients with chronic obstructive pulmonary disease (COPD) and analyzed them using the microimmunofluorescence assay and real-time PCR; however, only 3/210 (2.7%) patients were APMV-seropositive and none of the patients were positive for APMV DNA (Table 7). The authors suggested that the low seropositivity might be explained by either a low abundance of the virus or the presence of the virus may depend on the spatiotemporal regional variation as in the case with *Legionella*-caused infections [55], [56]. Negative PCR results could be explained by a number of reasons [55]. The viral load of the samples may have been below the detection limit of the PCR (12 copies/reaction), or a polymorphism in the area of amplification could occur [55]. A wide genomic diversity of *Mimiviridae* family may support the latter suggestion [19], [57], [55], [58], so patients could be positive for other viruses of the *Mimiviridae* family [55]. In addition, sputum is possibly not an appropriate study material compared to BAL sample [55]. In the second study performed by this research group with 260 bronchoalveolar lavage fluid samples from 214 Dutch patients suspected for VAP, no APMV DNA was detected in all of the samples using real-time PCR [59] (Table 7). Finally, in the recent study of Bousbia et al. [60], seroconversion to APMV was observed in 14/71 (19.7%) French pneumonia patients (ICU) with paired serum samples (Table 7). The authors also observed an elevation of the antibody response (both IgG and IgM) to APMV antigens in the convalescent-phase sera in comparison with the admission sera (16/41 (39.0%) patients with HAP and 4/29 (13.8%) controls (ICU patients without pneumonia), respectively, \( P=0.02 \) [60].

There are several explanations for the inconsistency observed between the results of the above-mentioned studies. The most evident explanation is that *Mimiviridae* family DNA detection could possibly have been hampered in studies that used genomic methods of detection. PCR primers used in these studies targeted only APMV genome, whilst a number of APMV relatives exhibiting considerable genetic diversity have been described (Table 3). Recently developed modern real-time PCR systems targeting giant viruses and their virophages are able to accurately detect all or most of the members of the currently delineated lineages of giant viruses infecting *Acanthamoeba* as well as the virophages [61]; hence, this obstacle may be overcome in the near future. Other reasons for the disparities may include differences in prevalence of giant viruses in distinct populations along...
Table 6: Cases of human infections caused by giant viruses

| Year and country | Virus                                      | Case description                                                                 | Sample type                      | Method of diagnostics                                                                 |
|------------------|--------------------------------------------|----------------------------------------------------------------------------------|----------------------------------|---------------------------------------------------------------------------------------|
| 2006, France [33]| Family Mimiviridae, lineage A, Acanthamoeba polyphaga Mimivirus                   | 28-year-old laboratory technician who handled large amounts of Mimivirus to perform Western blot assays with suspected pneumonia | Serum                            | Seroconversion to Mimivirus (against 23 Mimivirus proteins, including four unique proteins) |
| 2011, France [32]| Family Mimiviridae, lineage A, Lentiviruses                                        | 17-year-old myopic woman suffering from keratitis with soft contact lenses       | Contact lens storage case liquid  | Culture isolation on Acanthamoeba spp.                                                 |
| 2012, France [28]| Sputnik virophage of Acanthamoeba polyphaga mimivirus (family Mimiviridae, lineage A) | 29-year-old woman and her 32-year-old husband suffering from asthenia, nausea, myalgia, low-grade fever | Serum                            | Seroconversion to virophage                                                                |
| 2012, 2013, Senegal [34], [35]| Family Marseilleviridae, Senegavirus                                                  | 20-year-old Senegalese man living in rural Senegal. No clinical symptoms          | Stool                            | Metagenomics, culture isolation on Acanthamoeba spp.                                     |
| 2013, France [36]| Family Marseilleviridae, Giant blood Marseillevirus                                  | blood donor, no clinical symptoms                                                 | Serum                            | Metagenomics, PCR, culture isolation on lymphocyte T cells, transmission electron microscopy, fluorescence in situ hybridization |
| 2013, Tunisia [31]| Family Mimiviridae, lineage C, LBA111                                                | 72-year-old Tunisian woman with pneumonia                                         | Bronchoalveolar fluid            | Culture isolation on Acanthamoeba spp., serology                                        |
| 2013, Tunisia [37]| Family Mimiviridae, lineage C, Shan virus                                            | 17-year-old girl suffering from pneumonia                                         | Stool                            | Culture isolation, serology                                                              |
| 2013, France [38]| Family Marseilleviridae, Marseillevirus                                                | 11-month-old child with adenitis                                                   | Blood and stool                  | Fluorescence in situ hybridization (FISH), serology, PCR                                |

with spatiotemporal regional variation as well as the use of inappropriate study material (for instance, samples from upper respiratory tract instead of bronchoalveolar lavage (BAL) specimens). In some cases, giant viruses possibly could not be identified due to the too small viral load for the PCR detection in some cases, insufficient amount of specimens. In addition, APMV was shown to contain up to 23 proteins that may cause immune response resulting in a production of antibodies [33], so proteins of other Mimiviridae family members may cross-react with those of APMV [71]. In this case, molecular detection of APMV could be negative [62]. Exposure to APMV, since it is an AAM, most likely occurs from environmental sources such as contaminated hospital water supplies [52], which is the case in ICU patients. The high rate of seroconversion in pneumonia patients in certain studies [47], [49], [53] suggests that they may have had a contact with APMV or a cross-reacting agent which could possibly be other member of Mimiviridae family.

Conclusion

Viruses belonging to Mimiviridae family can be potential agents of both CAP and HAP. In particular, these viruses may be associated with poorer outcome in ICU patients. The exact mechanism of their pathogenicity, however, still remains unclear. The discrepancies between the results obtained by serological and genomic methods could be explained by the high polymorphism of nucleotide sequences of Mimiviridae family representatives. Further investigations on the Mimiviridae pathogenicity and on the determination of Mimiviridae-caused pneumonia risk groups are required. However, pathogenicity of the viruses belonging to Marseilleviridae family is unclear.
### Table 7: Studies on the association of the giant viruses with infections of the respiratory tract

| Year and country | Sample type | Method of detection | Number and share of cases and controls | Odds ratio (OR) with 95% confidence interval (95% CI) and P-value |
|------------------|-------------|---------------------|----------------------------------------|---------------------------------------------------------------|
| 2005, Canada     | Serum       | Micro-immuno/fluorescence between acute-phase and convalescent-phase samples | 376 patients with community-acquired pneumonia (CAP) | P=0.10, no significant                                           |
| 2005, France     | Serum       | Micro-immuno/fluorescence between acute-phase and convalescent-phase samples | 1251 controls (2.3%) | P=0.01, no significant                                           |
| 2014, Ireland    | Bronchial mucociliary clearance specimens | Micro-immuno/fluorescence between acute-phase and convalescent-phase samples | 157 patients with HAP (102 controls) | P=0.01, no significant                                           |
| 2016, France     | Nasopharyngeal aspirates | Molecular detection Real-Time PCR | 214 patients with suspected acute respiratory tract infections | Not detected |
| 2016, Austria    | Nasopharyngeal aspirates | Molecular detection Real-Time PCR | 477 specimens from 315 acute respiratory tract infections | Not detected |

**Notes:**
- GMS: Génétique Médicale des Virus
- Hygiene and Infection Control: 2014, Vol. 9(2), ISSN 2196-5226
- Kutikhin et al.: Mimiviridae, Marseilleviridae, and virophages as emerging...
| Year and country | Sample type | Method of detection                      | Number of cases and controls | Number and share of positive cases and controls | Odds ratio (OR) with 95% confidence interval (95% CI) and P-value |
|-----------------|-------------|------------------------------------------|------------------------------|-----------------------------------------------|---------------------------------------------------------------|
| 2008, USA, Canada, Thailand [52] | Nasal wash and swabs, nasopharyngeal swabs and aspirates, oro-pharyngeal swabs, BAL samples, sputum, endotracheal aspirates, lower respiratory samples | Molecular detection Real-Time PCR | 496 patients with pneumonia, 249 patients with CAP, 71 patients with HAP, 87 bone marrow transplant recipients with pneumonia, 89 lung transplant recipients with pneumonia | 0/496 (0.0%) | Not detected |
| 2009, France [53] | Serum | Micro-immuno-fluorescence 1) Seroconversion from <1:50 to ≥1:100 between acute-phase and convalescent-phase serum samples 2) 4-fold rise in antibody titer between acute-phase and convalescent-phase serum samples 3) Single or stable titer ≥1:400 | 55 seropositive patients with suspicion of VAP, 55 seronegative patients (ICU) | 59/300 patients with suspected VAP (19.6%) | Patients with a positive serology for mimivirus had longer duration of mechanical ventilation and ICU stay with median excesses of 7 days and 10 days, respectively |
| 2012, Italy [54] | BAL samples | Molecular detection Real-Time PCR | 30 patients on mechanical ventilation and 39 nonventilated patients | 0/69 (0.0%) | Not detected |
| 2012, Netherlands [55] | Sputum, serum | Micro-immuno-fluorescence 1) Seroconversion from <1:50 to ≥1:100 between acute-phase and convalescent-phase serum samples 2) 4-fold rise in antibody titer between acute-phase and convalescent-phase serum samples 3) Single or stable titer ≥1:400 Molecular detection Real-Time PCR | 220 sputum samples from 109 patients with chronic obstructive pulmonary disease (COPD) | 3/109 (2.7%) according to the micro-immuno-fluorescence assay 0/109 (0.0%) according to real-time PCR | Almost not detected |
| 2012, Sweden [41] | Nasopharyngeal aspirates | Molecular detection Metagenomic sequencing | 210 patients with lower respiratory tract infections | 2/210 (0.95%) | Almost not detected |
| Year and country | Sample type | Method of detection | Number of cases and controls | Number and share of positive cases and controls | Odds ratio (OR) with 95% confidence interval (95% CI) and P-value |
|------------------|-------------|---------------------|------------------------------|-----------------------------------------------|---------------------------------------------------------------|
| 2013, France [60] | Serum       | Micro-immuno-fluorescence  
1) Seroconversion from <1:50 to ≥1:100 between acute-phase and convalescent-phase serum samples  
2) 4-fold rise in antibody titer between acute-phase and convalescent-phase serum samples  
3) Single or stable titer ≥1:400 | 88 patients with pneumonia (ICU):  
55 patients with VAP,  
17 patients with CAP,  
8 patients with aspiration pneumonia (AP),  
8 patients with non-ventilator ICU-acquired pneumonia.  
29 controls from ICU | Both IgG and IgM:  
9/88 cases (10.2%)  
IgG: 1/29 controls (3.45%)  
IgM: 3/29 controls (10.34%)  
In acute phase sera:  
55 patients with VAP – IgG:  
5/55 (9.1%)  
IgM: 7/55 (12.7%)  
17 patients with CAP – both IgG and IgM: 2/17 (11.8%)  
8 patients with AP – both IgG and IgM: 0/8 (0.0%)  
8 patients with non-ventilator ICU-acquired pneumonia –  
IgG: 2/8 (25%)  
IgM: 0/8 (0.0%)  
In convalescent phase sera:  
IgG: 7/41 cases with HAP (17.1%)  
IgM: 9/41 cases with HAP (22.0%)  
In controls:  
IgG: 1/29 controls (3.4%)  
IgM: 3/29 controls (10.3%)  
Seroconversion:  
14/71 (19.7%) patients (ICU) with paired serum samples,  
9/48 (18.7%) patients with VAP,  
3/9 (33.3%) patients with CAP,  
2/14 (14.3%) controls | Antibody response (both IgG and IgM) was increased in convalescent phase sera compared to control sera  
P=0.02 |
| 2013, Netherlands [59] | BAL samples | Molecular detection  
Real-time PCR | 260 bronchoal-veolar lavage fluid samples from 214 patients suspected for VAP | 0/260 (0.0%) | Not detected |
Notes

Competing interests

The authors declare that they have no competing interests.

Funding

There was no funding for this paper.

References

1. Donelli G, Gore D, Frontali C, Grandolfo ME. Structure and physico-chemical properties of bacteriophage G. III. A homogeneous DNA of molecular weight 5 times 10^8. J Mol Biol. 1975 Jun 5;94(4):555-65. DOI: 10.1016/0022-2836(75)90321-6

2. Van Etten JL, Meints RH, Kuczmarski D, Burbank DE, Lee K. Viruses of symbiotic Chlorella-like algae isolated from Paramecium bursaria and Hydra viridis. Proc Natl Acad Sci USA. 1982 Jun;79(12):3867-71. DOI: 10.1073/pnas.79.12.3867

3. Raoult D, La Scola B, Birtles R. The discovery and characterization of Mimivirus, the largest known virus and putative pneumonia agent. Clin Infect Dis. 2007 Jul;45(1):95-102. DOI: 10.1086/518608

4. Greub G, Raoult D. Microorganisms resistant to free-living amoebae. Clin Microbiol Rev. 2004 Apr;17(2):413-33. DOI: 10.1128/CMR.17.2.413-433.2004

5. Barker J, Brown MR. Trojan horses of the microbial world: protozoa and the survival of bacterial pathogens in the environment. Microbiology (Reading, Engl). 1994 Jun;140(Pt 6):1253-9. DOI: 10.1099/00221287-140-6-1253

6. La Scola B, Audic S, Robert C, Jungang L, de Lamballerie X, Drancourt M, Birtles R, Claverie JM, Raoult D. A giant virus in amoebae. Science. 2003 Mar;299(5615):2033-6. DOI: 10.1126/science.1081867

7. Raoult D. Giant viruses from amoebae in a post-Darwinist viral world. Intervirology. 2010;53(5):251-3. DOI: 10.1159/000312909

8. La Scola B, Birtles R, Greub G, Harrison T, Ratcliffe RM, Raoult D, Legionella drancourti sp. nov., a strictly intracellular amoebal pathogen. Int J Syst Evol Microbiol. 2004 May;54(Pt 3):699-703. DOI: 10.1009/ijsm.0.02455-0

9. Adeleke AA, Fields BS, Benson RF, Daneshvar MI, Pruckler JM, Ratcliffe RM, Harrison TG, Weisheit RS, Birtles RJ, Raoult D, Halablab MA, Legionella drancourti sp. nov., Legionella rowbothamii sp. nov. and Legionella fallonii sp. nov.: three unusual new Legionella species. Int J Syst Evol Microbiol. 2001 May;51(Pt 3):1151-60. DOI: 10.1009/ijsm.0.027713-15-1151

10. Raoult D, Audic S, Robert C, Abergel C, Renesto P, Ogata H, La Scola B, Suzan M, Claverie JM. The 1.2-megabase genome sequence of Mimivirus. Science. 2004 Nov;306(5700):1344-50. DOI: 10.1126/science.1101485

11. Raoult D, Forterre P. Redefining viruses: lessons from Mimivirus. Nat Rev Microbiol. 2008 Apr;6(4):315-9. DOI: 10.1038/nrmicro1858

12. Forterre P. Giant viruses: conflicts in revisiting the virus concept. Intervirology. 2010;53(5):362-78. DOI: 10.1159/000312921

13. Iyer LM, Aravind L, Koonin EV. Common origin of four diverse families of large eukaryotic DNA viruses. J Virol. 2001 Dec;75(23):11720-34. DOI: 10.1128/JVI.75.23.11720-11734.2001

14. Iyer LM, Balaji S, Koonin EV, Aravind L. Evolutionary genomics of nucleo-cytoplasmic large DNA viruses. Virus Res. 2006 Apr;117(1):156-84. DOI: 10.1016/j.virusres.2006.01.009

15. Yutin N, Wolf YI, Raoult D, Koonin EV. Eukaryotic large nucleo-cytoplasmic DNA viruses: clusters of orthologous genes and reconstruction of viral genome evolution. Virol J. 2009;6:223. DOI: 10.1186/1743-422X-6-223

16. Koonin EV, Yutin N. Origin and evolution of eukaryotic large nucleo-cytoplasmic DNA viruses. Intervirology. 2010;53(5):284-92. DOI: 10.1159/000312913

17. Boyer M, Yutin N, Pagnier I, Barrassi L, Fournas G, Espinosa L, Robert C, Azza S, Sun S, Ross mann MG, Suzan-Monti M, La Scola B, Koonin EV, Raoult D. Giant Marseillevirus highlights the role of amoebae as a melting pot in emergence of chimeric microorganisms. Proc Natl Acad Sci USA. 2009 Dec;106(51):21848-53. DOI: 10.1073/pnas.0911354106

18. Fischer MG, Allen MJ, Wilson WH, Suttle CA. Giant virus with a remarkable complement of genes infects marine zooplankton. Proc Natl Acad Sci USA. 2010 Nov;107(45):19508-13. DOI: 10.1073/pnas.1007615107

19. Colson P, de Lamballerie X, Fourroux G, Raoult D. Reclassification of giant viruses composing a fourth domain of life in the new order Megavirales. Intervirology. 2012;55(5):321-32. DOI: 10.1159/000336562

20. Colson P, De Lamballerie X, Yutin N, Asgari S, Bigot Y, Bideshi DK, Cheng XW, Federici BA, Van Etten JL, Koonin EV, La Scola B, Raoult D. “Megavirales”, a proposed new order for eukaryotic nucleocytoplasmic large DNA viruses. Arch Virol. 2013 Dec;158(12):2517-21. DOI: 10.1007/s00705-013-1768-6

21. Boyer M, Madoui MA, Gimenez G, La Scola B, Raoult D. Phylogenetic and phyloetic studies of informational genes in genomes highlight existence of a 4 domain of life including giant viruses. PLoS ONE. 2010;5(12)e15530. DOI: 10.1371/journal.pone.0015530

22. Koonin EV, Senkevich TG, Dolja V. The ancient Virus World and evolution of cells. Biol Direct. 2006;1:29. DOI: 10.1186/1745-6150-1-29

23. Pagnier I, Reteno DG, Saadi H, Boughalmi M, Gaia M, Slimani N, Kutikhin et al.: Mimiviridae, Marseilleviridae, and virophages as emerging ...
59. Vanspauwen MJ, Schnabel RM, Bruggeman CA, Drent M, van Mook WN, Bergmans DC, Linssen CF. Mimivirus is not a frequent cause of ventilator-associated pneumonia in critically ill patients. J Med Virol. 2013 Oct;85(10):1836-41. DOI: 10.1002/jmv.23655

60. Bousbia S, Papazian L, Saux P, Forel JM, Auffray JP, Martin C, Raoult D, La Scola B. Serologic prevalence of amoeba-associated microorganisms in intensive care unit pneumonia patients. PLoS ONE. 2013;8(3):e58111. DOI: 10.1371/journal.pone.0058111

61. Ngouna T, Pagnier I, Reteno DG, Raoult D, La Scola B, Colson P. Real-time PCR systems targeting giant viruses of amoebae and their virophages. Intervirology. 2013;56(6):413-23. DOI: 10.1159/000354563

62. Vincent A, La Scola B, Papazian L. Advances in Mimivirus pathogenicity. Intervirology. 2010;53(5):304-9. DOI: 10.1159/000312915

63. Thomas V, Bertelli C, Collyn F, Casson N, Telenti A, Goesmann A, Croxatto A, Greub G. Lausannevirus, a giant amoebal virus encoding histone doublets. Environ Microbiol. 2011 Jun;13(6):1454-66. DOI: 10.1111/j.1462-2920.2011.02446.x

64. La Scola B, Barrassi L, Raoult D. Isolation of new fastidious alpha Proteobacteria and Afipia felis from hospital water supplies by direct plating and amoebal co-culture procedures. FEMS Microbiol Ecol. 2000 Dec;34(2):129-137.

65. Gaia M, Pagnier I, Campocasso A, Fournous G, Raoult D, La Scola B. Broad spectrum of mimiviridae virophage allows its isolation using a mimivirus reporter. PLoS ONE. 2013;8(4):e61912. DOI: 10.1371/journal.pone.0061912

66. Popgeorgiev N, Boyer M, Fancello L, Monteil S, Robert C, Rivet R, Nappé C, Azza S, Chironi J, Raoult D, Desnues C. Marseillevirus-like virus recovered from blood donated by asymptomatic humans. J Infect Dis. 2013 Oct;208(7):1042-50. DOI: 10.1093/infdis/jit292

67. Franzman PD, Deprez PP, Burton HR, van den Hoff J. Limnology of Organic Lake, Antarctica, a meromictic lake that contains high concentrations of dimethyl sulfide. Aust J Mar Freshw Res. 1987;38(3):409–17. DOI: 10.1071/MA9870409

68. Zhou J, Zhang W, Yan S, Xiao J, Zhang Y, Li B, Pan Y, Wang Y. Diversity of virophages in metagenomic data sets. J Virol. 2013 Apr;87(8):4225-36. DOI: 10.1128/JVI.00398-12

69. Clingenpeel S, Macur RE, Kan J, Inskeep WP, Lovaldo D, Varley J, Mathur E, Nealon K, Gorbey Y, Jiang H, LaFranois T, McDermott TR. Yellowstone Lake: high-energy geochemistry and rich bacterial diversity. Environ Microbiol. 2011 Aug;13(8):2172-85. DOI: 10.1111/j.1462-2920.2011.02466.x

70. Coolen MJ, Hopmans EC, Rijpstra WIC, Muyzer G, Schouten S, Volkman JK, Sinninghe Damsté JS. Evolution of the methane cycle in Ace Lake (Antarctica) during the Holocene: response of methanogens and methanotrophs to environmental change. Org Geochem. 2004 Oct;35(10):1151–67. DOI: 10.1016/j.orggeochem.2004.06.009

71. Bartlett JG, Dowell SF, Mandell LA, File TM Jr, Musher DM, Fine MJ. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. Clin Infect Dis. 2000 Aug;31(2):347-82. DOI: 10.1086/313954

Corresponding author:
Dr. Anton G. Kutikhin
Department of Epidemiology, Kemerovo State Medical Academy, Darvina Street 2 – 9, Kemerovo 650025, Russian Federation, Phone: +73842751744, Fax: +73842751744
antonkutikhin@gmail.com

Please cite as
Kutikhin AG, Yuzhalin AE, Brusina EB, Mimiviridae, Marseilleviridae, and virophages as emerging human pathogens causing healthcare-associated infections. GMS Hyg Infect Control. 2014;9(2):Doc16. DOI: 10.3205/dgkh000236, URN: urn:nbn:de:0183-dgkh000236

This article is freely available from http://www.egms.de/en/journals/dgkh/2014-9/dgkh000236.shtml

Published: 2014-08-19

Copyright
©2014 Kutikhin et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by-nc-nd/3.0/deed.en). You are free: to Share — to copy, distribute and transmit the work, provided the original author and source are credited.