Isolation and Quantification of Mimivirus-Like and Marseillevirus-Like Viruses from Soil Samples in An Aboriginal (Orang asli) Village in Peninsular Malaysia

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Keywords

Mimivirus · Orang asli · Marseillevirus

Abstract

Background: The giant amoebal viruses of Mimivirus and Marseillevirus are large DNA viruses and have been documented in water, soil, and sewage samples. The trend of discovering these giant amoebal viruses has been increasing throughout Asia with Japan, India, and Saudi Arabia being the latest countries to document the presence of these viruses. To date, there have been no reports of large amoebal viruses being isolated in South East Asia. **Objective:** In this study, we aim to discover these viruses from soil samples in an aboriginal village (Serendah village) in Peninsular Malaysia. **Method and Results:** We successfully detected and isolated both Mimivirus-like and Marseillevirus-like viruses using *Acanthamoeba castellanii*. Phylogeny analysis identified

them as Mimivirus and Marseillevirus, respectively. **Conclusion:** The ubiquitous nature of both Mimivirus and Marseillevirus is further confirmed in our study as they are detected in higher quantity in soil that is near to water vicinities in an aboriginal village in Peninsular Malaysia. However, this study is limited by our inability to investigate the impact of Mimivirus and Marseillevirus on the aboriginal villagers. More studies on the potential impact of these viruses on human health, especially on the aborigines, are warranted.

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Background

Giant amoebal viruses which were discovered over a decade ago comprised of 2 distinct families: Mimiviridae and Marseilleviridae. Viruses of the family Mimiviridae had been isolated and characterized with increasing fre-

Fig. 1. Sampling sites at Serendah aboriginal village and its surrounding areas in sketch (**a**) and Google map (**b**). SR1–3, site of the waterfall (SR2 is midpoint between SR1 and SR3; not tagged in the map); SR4, midpoint between waterfall and village; SR5, village health clinic center located at top of a slope leading to the bank for

a stream; SR6, football field; SR7, near a gravity toilet at top of a slope leading to the bank for the same stream; SR8, ex-construction site situated at an inclination; SR9, ex-agricultural land situated next to a stream opening out to a lake. (Adapted from Google Maps.)

quency recently in Asian countries such as Japan, India, and Saudi Arabia [1–3]. However, at the time of writing, there were no reports of giant amoebal viruses detected in South East Asia. Due to the ubiquitous nature of giant amoebal viruses, both Mimivirus and Marseillevirus have been found in a wide range of environments. These include water samples from man-made structures like cooling tower and from natural sources like river, lake, or ocean [4–9]. They are also found in soil, on other animals like leech and oyster, and disturbingly, some on human samples [10–15]. In this paper, we describe the isolation and distribution both Mimivirus-like and Marseillevirus-like viruses from the Serendah Village, which is an aboriginal settlement in Peninsular Malaysia.

Nine sampling points (SR1–9) were tagged across Serendah village (Central Peninsular Malaysia; Fig. 1). For each sampling point, 9 samples were collected within a 500-m radius of the point. These samples were pooled, transported in cold chain, and subsequently stored at -80°C . Isolation of Mimivirus or Marseillevirus was performed through blind passages on *Acanthamoeba castellanii* (ATCC 30010) until cytopathic effects (CPE) were observed. Infected *Acanthamoeba castellanii* were then Giemsa-stained to detect the viral factories. DNA was extracted from the pooled soil samples using TIANamp soil DNA kit (TIAN, China) according to the manufacturer's protocol. In those samples where Mimivirus-like or Mar-

seillevirus-like viruses were successfully isolated, phylogeny was performed by using specific primers targeting the helicases of Mimivirus and Marseillevirus as previously described by Slimani et al. [16, 17]. DNA sequences were deposited in the Genbank (MG981284–7). Quantification of Mimivirus and Marseillevirus partial viral genome copies in the soil samples was performed as previously described by Ngounga et al. [18].

Out of the 9 sampling sites, 4 of them (SR1, 4, 5, and 9) were demonstrated to have CPE on the cultured *Acanthamoeba castellanii* after several rounds of blind passages. Viral inclusion factories were spotted in these cultures with CPE that were stained with Giemsa stain, indicating Mimivirus-like infection (see online suppl. Fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000491602). Further molecular identification through the construction of phylogenetic tree showed that Mimiviruses were detected on SR1, 4, and 9. The virus detected in SR5 belonged to Marseillevirus (Fig. 2). Quantification of the Mimivirus from all 9 sampling sites showed that these viruses were present in high levels in the respective soils where Mimiviruses were isolated (SR1, 4, and 9) and not in other sampling sites. Similarly, Marseillevirus was detected at a high level in SR5 (Fig. 3).

Although the impact of these viruses to the health of the Serendah village inhabitants is not known yet, there are of increasing evidences supporting that Mimiviruses

Fig. 2. Phylogenetic tree demonstrating genetically close-relatedness of isolates SR1, 4, 9 (a), and SR5 (b) to Mimivirus and Mar-
seillevirus, respectively. Neighbor joining
tree was performed on helicase gene with
bootstrap of 1,000 replicates. Accession
numbers were stated on each taxa.

Fig. 3. Quantification of Mimivirus and Mar-
seillevirus from soil samples. SR1, SR4,
and SR9 showed higher Mimivirus DNA
while SR5 showed higher Mar-
seillevirus DNA compared to other sampling sites.
ND, not detectable.

and Mar-
seillevirus are infectious agents in humans. These
giant viruses had been isolated and detected from clinical
specimens from patients as well as healthy individuals
[15, 19]. In addition, Mimivirus was detected in domestic
animals such as *Bos taurus* and evidences of neutralizing
antibodies have been reported in farmers handling do-
mestic animals, indicating the risk of exposure of Mimi-
viruses from the animals [20].

This study also showed that spatial distribution of
Mimivirus-like and Mar-
seillevirus-like viruses in the
Serendah village differ among the collection points espe-

cially where water source is within the vicinities. The data
indicated that viral titers were higher in soils (SR1, SR4,
SR5, and SR9) which are near the water source and lower
in samples further away from the water sources. Mar-
seillevirus-like virus was isolated from soils collected at the
vicinity of the health clinic (SR5), located on top of the
slope leading to the bank of a stream. Similarly, Mimi-
virus-like virus was isolated from soils collected at the water
fall (SR1), midway to the water fall (SR4), and ex-agricul-
tural land (SR9) situated next to a stream opening out to
a lake. To conclude, viruses of Mimiviridae were discov-

ered in Peninsular Malaysia further supporting the ubiquitous nature of the Mimivirus and Marseillevirus. This study warrants a further investigation on the circulation and epidemiology of Mimiviridae in aboriginal settlements in Malaysia.

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Availability of the Data

All data generated or analyzed during this study are included in this published article and its online supplementary Figure and Table.

Ethics Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Authors Consents

All authors consent for publication.

Disclosure Statement

All authors declare non-competing interest.

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Author's Contribution

K.V. and C.W.C. designed the experiments; Y.F.T. and C.Y.L. contributed to sample processing, viral DNA extraction, PCR and subcloning; K.V. performed the viral culture. PLKC provided the helminths egg counts data. P.P.L. and K.V. provided essential reagents and helped write the paper; C.W.C. provided valuable suggestion. Y.F.T. and K.V. revised the paper. All authors read and approved the final manuscript.