

Commentary – Zombie viruses: genomic properties, molecular morphology and its biothreat

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New viruses are often emerging – and re-emerging – and are often considered to be a serious public health threat. Known viruses like, smallpox virus and *Influenza A* H1N1 virus have been isolated from old frozen specimens from the permafrost. H1N1 RNA was retrieved from lung tissue of a Spanish flu victim buried within the permafrost¹. Two wooden mummies that date to the 17th and 18th century were discovered in the Siberian tundra during an archaeological discovery. Much later in 2014, scientists were able to isolate the genome of smallpox virus, which is caused by *Poxviridae* family member *Variola virus*, the causative agent for smallpox².

Unusual viruses that had been kept for more than 45,800 years at extremely low temperatures have been analyzed in a recent article³. These are a diverse group of viruses with different morphology, protein structure, and nucleic acid composition. They were retrieved from thawing permafrost from places adjacent to the north pole. The size of these viruses was unusually large, ranged from 500 to 1500 nm, thus becoming the largest viruses ever found on the Earth. Since these viruses are larger than some bacteria, they can be visualized under a normal light microscope. These viruses were associated with infections of amoeba (*Acanthamoeba*) and other protists (Figure 1). Predominantly, these were eukaryotic viruses, isolated from higher living beings. These viruses are called “zombie viruses” because

they were preserved in permafrost from the Paleolithic era and possess the potential to resuscitate them. Several of these viruses were identified and grouped under several families and genera. They are *Pandoravirus*, *Pithovirus sibericum*, *Pacmanvirus lupus*, *Megavirus mammoth*, *Cedratvirus*, *Pithoviridae*, *Orpheoviridae*, *Megaviricetes*, *Pokkesviricetes* and *Nucleocyotoviricota*.

Climate change definitely plays a crucial role in bringing out these Paleolithic viruses to modern 21st century. Since these viruses affects eukaryotic organisms, its current ability to cause diseases in living organisms on the Earth is not ruled out⁴. It is crucial to identify host specificity of zombie viruses. These viruses may have a broad range of host heterogeneity. Scientific data are not currently available on the pathogenicity and virulence of these viruses. Viruses of modern era, like zoonotic viruses, arboviruses, rodent borne viruses etc., have the capacity to transfer from one host to another resulting in outbreaks and epidemics. The possibility of similar epidemiological patterns cannot be excluded for zombie viruses. Zombie viruses might be having the potential to cause different types of infections, particularly fatal diseases. Since our medical personnel and researchers are not prepared for the diseases caused by zombie viruses, and outbreak of this nature may land up in a catastrophe. This article brings the current update on zombie viruses.

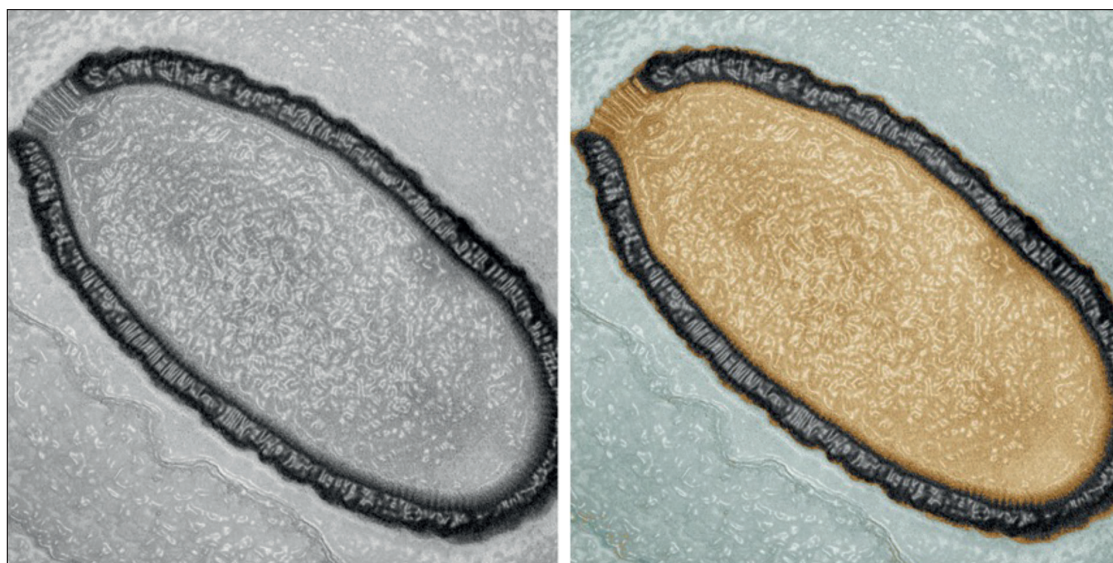


Figure 1. Electron microscope of *Pithovirus sibericum* (image credit: Juliabartoli and Chantal Abergel, IGS and CNRS-AMU).

Morphology of Zombie virus

These viruses are oval in shape covered by a tegument. Some zombie viruses possess capsid whereas others do not have. These viruses are generally pleomorphic and their size generally more than 1000 μm . The whole viral genome consists of 25 to 2,500 genes. Some zombie viruses like *Pandoravirus* have ‘amphora shaped’ structure with pointed ends. *Mimivirus* have icosahedral (cubic) symmetry with five-pronged star like morphology. Many zombie viruses have elongated, circular or slightly flattened on one side.

How These Viruses Were Exposed?

Permafrost, or permanently frozen ground, covers one-fourth of the Northern Hemisphere. It is capable of preserving and hosting a wide range of centuries old microorganisms, including known and unknown viruses. Permafrost is irrevocably thawing as a result of global warming, releasing organic material that has been frozen for up to a million years. Prokaryotes and unicellular eukaryotes, as well as viruses that have been dormant since the dawn of time, are also included in this organic material.

Permafrost microbes, which are permanently frozen in paleo soils, are the subject of growing investigation in relation to global warming. Two enormous viruses that infect *Acanthamoeba*,

Mollivirus sibericum and *Pithovirus sibericum*, were discovered and reactivated in a 30,000-year-old permafrost layer⁵. Although multiple contemporary *Pithovirus* strains have been discovered, no modern *Mollivirus* like viruses have been discovered so far. There is another zombie virus named as *Mollivirus kamchatka*, a closely related to *Mollivirus sibericum* that was discovered in surface soil samples taken in Kamchatka, Russian Federation, along the bank of the Kronotsky River. This finding revealed that molliviruses are still viable, active and exist in a remote subarctic continental region. The nucleocytoplasmic replication cycle of this contemporary viral isolate is similar to *Mollivirus sibericum* virus cycle.

Were They Associated with The Diseases of Mammoths and Neanderthals?

A zombie virus called *Megavirus mammoth* found to be associated with mammoths. *Megavirus mammoth* belongs to the family of *Megavirinae* that includes many giant viruses of mammals of Paleolithic. During Paleolithic era, Neanderthals were distributed in different parts of the Earth. We hypothesize that Neanderthals might had acquired zombie virus diseases from animals or from the Paleolithic environment. Extensive studies are required on isolation and recovery of zombie viruses from remnants of Neanderthals in Northern Hemisphere or other frozen areas⁶.

Genome and Molecular Properties of Zombie Viruses

Giant viruses are from the phylum *Nucleocytoviricota* are a diverse group of double stranded DNA viruses (dsDNA). *Pithoviridae* and *Orpheoviridae*-like families followed by *Mimiviridae* are the main viruses of the giant virus diversity of the deep permafrost. Scientists were able to assemble a complete 1.6Mb *Pithoviridae*-like circular genome from a 42,000-y-old sample⁷. Permafrost viral sequences revealed a patchwork of predicted functions amidst a larger reservoir of genes of unknown functions. The phylogenetic reconstructions revealed gene transfers between cells and viruses and between viruses from different families. The size of the virion is 1.5 µm which is comparable to sizes of many cellular lineages. The viruses of this family will have large genomes that are often >300 kbp in length, and in some cases as large as 2.5 Mbp. The possibility of release of dormant unknown eukaryotic viruses from the permafrost poses a possible danger to plants, animals and humans. According to some authors, all zombie viruses have the ability to infect humans, which poses as a serious public health threat to mankind.

Conflict of Interest

The authors declare that they have no conflict of interests.

Ethics Approval

Not applicable.

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