



Future threat from the past

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Abstract

Global warming is one of the major challenges facing humanity. The increase in the Earth's temperature and thawing of ancient ice release viable viruses, bacteria, fungi, and other microorganisms which were trapped for thousands and millions of years. Such microorganisms may belong to novel microbial species, unknown genotypes of present pathogens, already eradicated pathogens, or even known pathogens that gained extremely robust characteristics due to their subjection to long-term stress. These worries drew more attention following the death of a child by ancient anthrax spores in Siberian in 2016 and the reconstruction of smallpox and Spanish flu genomes from ancient frozen biological samples. The present review illustrates some examples of recently recovered pathogens after being buried for millions of years, including some identified viable ancient viruses, bacteria and even other forms of life. While some pathogens could be revived, genomes of other ancient pathogens which could not be revived were re-constructed. The present study aims to highlight and alarm the hidden aspect of global warming on the international public health, which represents future threats from the past for humanity.

Keywords Emerging diseases · Global warming · *Mollivirus sibericum* · *Pithovirus sibericum*

Introduction

With the continuous evolution and emergence of new unknown pathogens in the last decades, such as SARS, MERS, Ebola, and recently SARS2 (Covid-19), it is clear that international public health faces severe challenges. However, the re-emergence of serious infectious diseases which were previously partially or completely eradicated represents a similar public health hazard. One of the main reasons for the re-occurrence of eradicated diseases is attributed to the global warming. Among the most underestimated harmful effects of the global warming is the melting of the ice layer and the release of buried materials since decades including radioactive wastes (Colgan et al. 2016), and liberates billions of tons of carbon dioxide and methane gas into the air (Knoblauch et al. 2018), and could even release a huge amount of trapped heavy metals to the surrounding environment and groundwater (McConnell et al. 2018).

Thawing of frozen snow may also liberate frozen biological materials since tens and hundreds of thousands of years, including ancient viruses and bacteria. Microbiological examination of tissue samples obtained from a frozen mammoth in Siberian revealed the presence of members of the genera *Carnobacterium* and *Lactosphaera*, which could be cultured on anaerobic media (El-Sayed and Kamel 2020; Pikuta et al. 2011). Similarly, examination of the gut microbiome of the frozen body of rhinoceros, which represents another extinct animal species, could detect the presence of *Firmicutes* (mainly members of the family *Clostridiaceae*), *Proteobacteria*, *Actinobacteria*, TM7, and *Bacteroidetes* (Mardanov et al. 2012). Ancient bacteria were also isolated from environmental samples rather than the bodies of frozen animals. The diversity of newly detected bacterial species in ice is huge. Climatic changes that may lead to thawing of ice and the revival of bacteria will have potential effects with unexpected consequences. (Brouchkov et al. 2017). A 300,000-years-old virus and 8 million years old bacteria could be isolated from Siberian and Antarctica, respectively. Fourteen bacterial isolates could also be isolated from 750,000-year-old ice samples obtained from the Tibetan Plateau. In 2016, ancient anthrax spores stored in frozen soil in Siberian resulted in the death of a child died and hospitalization of an additional 20 persons (El-Sayed and Kamel 2020; Christner et al. 2003). However, the melted snow also released unknown bacteria/viruses that

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were trapped and preserved for thousands and possibly millions of years. Microbiological investigation of ice samples obtained from the Tibetan Plateau revealed four types of known viruses in addition to 28 novel viral genera and abundant bacteria. Similarly, the investigation of frozen samples from Siberia described for the first time a 30,000-year-old giant virus. The virus retained its viability and infectivity (Legendre et al. 2015).

The international worry is not only limited to the revival of unknown ancient viruses but also pathogens which were already eradicated. Fatal pathogens as smallpox may re-emerge from a mummified corpse (Reardon 2014).

Various bacteria respond to environmental stress by entering a viable non-culturable state (VBNC) (McDougald et al. 1999), even the presence of killed/non-viable bacterial DNA in the melted ice represents a great threat according to the concept of Griffith's experiment. In 1928, Frederick Griffith mixed a heat-killed rough strain with a smooth strain of *Streptococcus pneumoniae* and injected the mixture in a mouse. The mouse died due to bacterial *transformation*. It is worth mentioning that killed microbes in glacial ice are very well preserved due to the sub-zero temperature by (Griffiths 2000). Moreover, the revived ancient bacteria were subjected to stress for many thousands and probably millions of years which improved their ability to survive and overcome unsuitable environmental conditions. Among the developed survival mechanisms of newly discovered bacteria is their ability to resist the damaging effect of antibiotics (Petrova et al. 2011; D'Costa et al. 2011).

Identification of viable ancient viruses

The high robustness of smallpox virus (variola) enabled the virus to survive under different unfavourable conditions. Remnants of smallpox DNA could be detected around the world. The viral DNA could be identified in skin lesions of the 3200-year-old mummy of Rameses V in Egypt (Reardon 2014).

In 1991, in a small village near the North Pole, Russian experts discovered a wooden vault which was full of frozen victims who died from smallpox in the nineteenth century during the smallpox epidemic there. This discovery arose the question about the possible comeback of smallpox following floods induced by glacial ice thawing due to global warming. This worry is justified by the robust properties of smallpox and its ability to withstand freezing for a long time (Stone 2002; Ambrose 2011; Biagini et al. 2012).

Although all attempts to isolate viable variola from preserved human scabs and corpses were not successful, the viral genome could be re-construct through sequencing the detected DNA fragments, which represents a huge threat for public health (Reardon 2014; Duggan et al. 2016). Like smallpox

virus, different influenza viruses are also known to persist natural freezing for a long time (Zhang et al. 2006). In 1997, several trials were attempted to revive the 1918 pandemic influenza virus from frozen samples. Although these trials failed to achieve their goal, they could partially sequence and characterize partial sequences of the restored RNA (Taubenberger 1997; Reid et al. 1999). The trials continued later with the development of reverse genetics technology where the virus could be completely re-constructed (Taubenberger et al. 2007, 2012). Additional unknown viruses could also be detected in a 700-year-old caribou frozen stool which was stored in a permanent ice layer. Two viruses (RNA and DNA viruses) could be recovered and characterized in two different laboratories from the frozen faecal sample (Ng et al. 2014).

One of the oldest viable viruses was detected in ice samples obtained from Greenland. Various genotypes of the plant virus (Tomato mosaic tobamovirus) could be identified in approximately 140,000-year-old ice layers. The highly conserved viruses are characterized by their high stability and their wide host range (Castello et al. 1999).

The newly discovered ancestral amoeba-infecting DNA viruses (*Pithovirus sibericum* and *Mollivirus sibericum* viruses) are estimated to be older than 30,000 years old. It belongs to an unknown type of giant viruses. They are among the largest viruses ever known and can even be seen under a light microscope. Although *Pithovirus sibericum* is 1.5 μm in length, its genome is relatively small (approximately 600 kb) packaged in uniquely amphora-like shaped particles. The virus kept its viability and infectivity (Legendre et al. 2014, 2015).

Another virus (*Emiliana huxleyi* virus) was also detected in 7000-year-old sediment samples obtained from the Black Sea. The obtained data confirmed the ability of the virus to survive hard environmental conditions for centuries (Coolen 2011).

As the viruses are defined as particles that are not alive outside the living organism, their survival over thousands of years was not a big surprise. The survival of ancient frozen bacteria over millions of years despite the hard environmental conditions was not expected to date. The adverse environmental conditions include not only the low temperature but also the absence of nutrients and sources of energy, in addition to the increase in the concentration of nascent oxygen at low temperature (which has a damaging effect on bacterial DNA /RNA and proteins). However, the bacteria could maintain their metabolic activities, could grow, and even could multiply through the development of novel metabolic survival strategies, such as methanogenesis in addition to their potential DNA repair mechanisms in association with high-stress tolerance (Lewis et al. 2008; Johnson et al. 2007; Vishnivetskaya et al. 2000; Tuorto et al. 2014; Hultman et al. 2015).

It is also worth mentioning that viable bacteria could be detected not only in frozen ice but also in ancient highly preserved fossils. *Bacillus sphaericus* and *Staphylococcus succinus* could be revived and cultured from 25 to 40 million-year-old Dominican amber (Greenblatt et al. 1999).

Identification of viable ancient bacteria

Ancient DNA fragments from various human pathogens could be extracted and identified. Some of these DNA fragments originated from the Bronze Age. Among the identified pathogens are DNA from *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Vibrio cholerae*, *Yersinia pestis*, and *Helicobacter pylori* (Margaryan et al. 2018).

Generally, the isolation of various gram-positive and gram-negative bacteria from ancient ice samples has been previously reported. In Siberian samples, the prevalence of gram-negative bacteria was much higher than gram-positive ones. The majority of these isolates were identified as *Arthrobacter* (*Actinobacteria*) and *Planococcus* (*Firmicutes*) (Shi et al. 1997; Hinsä-Leasure et al. 2010).

Few pockets of 34-million-year old ice layers in the Antarctic continent are still preserved. Investigation of the ice samples revealed the presence of viable metabolically active bacteria, which are estimated to be 8 million years old. These bacteria are among the oldest known living organisms on earth. The revival of the bacteria was only possible following long incubation at 4 °C in the dark. Sequencing of the DNA of the revived bacteria identified them as *Arthrobacter roseus* (Bidle et al. 2007). Relatively younger viable bacteria which are 3.5 million years could also be isolated from Eastern Siberia. Sample examination detected various archaea, phototrophic cyanobacteria, algae, fungi, and even protozoa in addition to heterogeneous bacterial species in the samples. The isolated bacteria belonged mainly to gram-negative members. The majority of isolates could be identified as *Arthrobacter phenanthrenivorans*, *Subtercola frigoramans*, and *Glaciimonas immobilis* (Zhang et al. 2013).

Meanwhile, viable and highly robust 3.5-million-year old strains of *Bacillus* sp., including *Bacillus cereus* and *Bacillus anthracis*, were reported (Nicholson et al. 2000; Fursova et al. 2012). The dominance of spore-forming bacteria in ancient Canadian ice samples was also reported. This is attributed to their ability to withstand hard environmental conditions (Steven et al. 2007).

On the other hand, microbiological examination of 3.5-million-year old samples from Eastern Siberia revealed the absolute dominance of microbial members of three phyla *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* (representing about 99% of the identified bacterial population). In contrast, the remaining 1% was represented by members of *Deinococcus-Thermus*, *Cyanobacteria/Chloroplast*,

Fusobacteria, and *Acidobacteria*. In turn, members of the families *Chitinophagaceae*, *Caulobacteraceae*, *Sphingomonadaceae*, *Bradyrhizobiaceae*, and *Halomonadaceae* clearly dominated over other microbes (Brouchkov et al. 2017).

Younger samples from Northeast Eurasia (approximately 3 million years old) were also examined. Characterization of the cultured bacteria revealed three major lineages: gamma-*Proteobacteria* (mainly *Xanthomonadaceae*), *Actinobacteria*, and *Firmicutes*. In addition, various aerobes could be isolated from the ice samples including *Actinomycetales* (*Arthrobacter* and *Microbacteriaceae*); *Firmicutes* (*Exiguobacterium* and *Planomicrobium*); *Bacteroidetes* (*Flavobacterium*); and finally alpha- and gamma-*Proteobacteria* represented by *Sphingomonas* and *Psychrobacter*, respectively (Vishnivetskaya et al. 2006). About 30% of the grown bacteria could build endospores. The isolated bacteria from the same location showed variation according to the age of the samples. In contrast, the older samples (up to 3 million years) revealed mainly high-GC gram-positive bacteria; in addition to beta- and gamma-*Proteobacteria*, younger samples from the region (up to 8000 years) contained mainly low-GC gram-positive bacteria (Shi et al. 1997). At the same time, the microbial diversity of younger samples clearly increased, while the prevalence of spore-forming bacteria in the microbial population decreased. The same findings were also observed in ice samples originating from Siberia, Canada, and Norway, where the spore-forming bacteria (mainly *Clostridia* and *Bacilli*) were more prevalent in ice layers (up to 33,000 years old), while non-spore-forming species dominated in older samples (about 600,000 years old) (Liang et al. 2019).

Identification of other forms of life

The melted ancient ice samples contain not only viruses and bacteria but also fungi, amoeba, nematodes, and even arthropods. In 2013, a mycological examination of ancient Siberian permafrost sediment detected different fungal species which were viable and metabolically active (Zhang et al. 2013).

Similarly, various viable amoebae strains representing unknown species from the genus *Flamella* (Amoebozoa, Varioseae) could be isolated from frozen Arctic samples. Although the amoebae cysts were conserved over thousands of years in frozen ice, they kept their viability and ability to divide (Shmakova et al. 2016). Similarly, examination of 30,000 to 40,000-year-old permafrost deposit samples could also detect viable nematodes (*Panagrolaimus* aff. *Detritophagus* (Rhabditida) and *Plectus* aff. *Parvus* (Plectida)) (Shatilovich et al. 2018). They were found to be very tolerant to hard environmental conditions and low temperatures up to – 80 °C (Kagoshima et al. 2012). In addition,

ice samples from Antarctica harboured freeze-tolerant arthropods such as the midges *Belgica antarctica* and *Eretmoptera murphyi* (Teets and Denlinger 2014).

In conclusion, the consequences of global warming on international public health are still underestimated. The climatic changes have now been shown to influence various aspects of human and animal health and represent a serious threat to the existence of humanity.

Authors' contribution AE and MK wrote the initial draft of the manuscript. All authors (AE and MK) have read, edited, and finally approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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