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Cooling perspectives on the risk of pathogenic viruses from thawing permafrost

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ABSTRACT Climate change is inducing wide-scale permafrost thaw in the Arctic and subarctic, triggering concerns that long-dormant pathogens could reemerge from the thawing ground and initiate epidemics or pandemics. Viruses, as opposed to bacterial pathogens, garner particular interest because outbreaks cannot be controlled with antibiotics, though the effects can be mitigated by vaccines and newer antiviral drugs. To evaluate the potential hazards posed by viral pathogens emerging from thawing permafrost, we review information from a diverse range of disciplines. This includes efforts to recover infectious virus from human remains, studies on disease occurrence in polar animal populations, investigations into viral persistence and infectivity in permafrost, and assessments of human exposure to the enormous viral diversity present in the environment. Based on currently available knowledge, we conclude that the risk posed by viruses from thawing permafrost is no greater than viruses in other environments such as temperate soils and aquatic systems.

KEYWORDS permafrost thaw, viruses, climate change, environmental microbiology

Permafrost underlies a quarter of Earth's terrestrial surface (1) and, despite millennia of subzero temperatures, hosts diverse microbial communities (2). Some microorganisms survive by altering their physiology and maintaining low rates of metabolic activity. Others adopt dormant forms such as endospores or cysts (3–7). Viruses that infect bacteria, archaea, and microeukaryotes (e.g., fungi and amoebae) are also abundant members of these microbial communities, both in thawing and intact permafrost (8, 9). They can survive by infecting active cells, through passive existence as prophages, or as "stowaways" when an infected cell enters dormancy. In some cases, cold-adapted viruses may persist frozen outside of their hosts (10–13). Permafrost also contains the remains of dead microbes, plants, animals, and occasionally humans, which are preserved by frozen conditions (3, 14, 15). Climate change may thaw up to 40% of Arctic and subarctic permafrost by the end of the century (16), exposing the preserved remains of infectious disease victims (both human and animal). If viral pathogens maintain infectivity while frozen in permafrost, or if permafrost viruses with microbial hosts can jump to humans or animals, thaw could potentially initiate disease outbreaks (17). However, there are many reasons why this possibility should not result in a heightened sense of alarm.

The possibility of pathogenic microorganisms emerging from thawing permafrost and causing disease is not completely without precedent. In 2016 an anthrax outbreak, caused by the bacterium *Bacillus anthracis*, resulted in the death of one person, sickened dozens more, and killed thousands of domesticated reindeer on the Yamal Peninsula in Russia (18). Prior to the introduction of a vaccine around 1930, anthrax was common on the Yamal Peninsula and periodically decimated reindeer populations (19). Reindeer herds likely acquired anthrax through the uptake of environmentally resistant endospores, which can remain dormant in the soil for decades. This is akin to transmission

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pathways in grazing animals from temperate regions (20). From ~1930 to 2016, anthrax was virtually absent on the Yamal Peninsula due to reindeer vaccination programs (19). However, routine vaccination was discontinued in 2007, leading to an immunologically naive host population that was susceptible to infection by *B. anthracis* (18, 21). It is unclear whether the 2016 outbreak was caused by *B. anthracis* acquired from thawing permafrost and/or thawed reindeer carcasses, or if it occurred independent of thaw in a region where anthrax was endemic (22). Vaccination has since resumed, and no further outbreaks have been reported (23).

The inability of anthrax to spread from person to person and the availability of modern vaccines and antibiotics (24) suggest that emergence of *B. anthracis* spores from thawing permafrost would be unlikely to cause a geographically widespread epidemic or pandemic. In general, modern antibiotics make potential emerging bacterial pathogens more containable than viral pathogens. We therefore focus on viral pathogens for the remainder of this perspective.

Potential viral pathogens from thawing permafrost could cause human disease outbreaks through three general pathways. The first is reintroduction of human viral pathogens, such as those causing influenza and smallpox, from thawing graves, mass

BOX 1. DEFINITIONS AND TERMINOLOGY

Viral pathogen

A virus that "causes damage in a susceptible host or has the potential to do so" (25).

Spillover

Transmission of a virus from one species to another, establishing an infection in a new host (25, 26). This definition includes spillovers that are followed by transmission between individuals in the new host species and spillovers that dead-end in the new host (i.e., there is no subsequent transmission between individuals in the new host species), as occurs when hantaviruses and the rabies virus spillover into humans from animal hosts (27, 28).

Host jump

We define host jump as a spillover followed by sustained transmission between individuals in the new host species.

Zoonosis

Infection transmitted from non-human animals to humans.

Giant viruses

The existence of giant viruses was first recognized in 2003 when a virus measuring 0.7 µm in diameter was isolated from an amoeba host (29). Since then, many additional giant viruses have been identified through cultivation-based approaches and in metagenomic sequence data from environmental samples (30–32). They are characterized by their exceptionally large size, can typically be observed with a light microscope, and are larger than some bacterial cells. Giant viruses have been placed in the diverse *Nucleocytoviricota* phylum, which includes other lineages of large (but not giant) viruses. There is not a formal consensus on which clades within *Nucleocytoviricota* constitute giant viruses, in part because taxonomic classification is challenging (33), but they typically include members of the *Mimiviridae*, *Marseilleviridae*, *Pithoviridae*, and *Pandoraviridae* families (31, 34–37). In some instances, the term "giant viruses" has been expanded to describe the entire *Nucleocytoviricota* phylum (38). However, this phylum covers an enormous phylogenetic range (including variola virus, which causes smallpox) with lineages that differ substantially from giant viruses found in permafrost and other environments (31, 39, 40). Here, we adhere to the non-expanded use of the term that differentiates between large and giant viruses.

burial sites, or soil or ice to the modern environment. The second is reintroduction of viruses with wildlife or domestic animal hosts from thawed remains or soil and ice, which could be transmitted directly to humans or could infect animals before jumping to humans. The third is the spillover of permafrost viruses with microbial hosts, such as microeukaryotes, bacteria, and archaea, to humans or animals when permafrost thaws. To initiate an outbreak, viruses spilling over would need to both establish an infection in the new host and subsequently be transmitted between individuals in the new host species. As discussed in more detail in later sections, this third pathway is an especially unlikely scenario. All of these general pathways depend on whether viruses can survive and remain infectious for decades to millennia in permafrost in sufficient numbers to cause disease. The latter two also depend on the ability of viruses to spillover from the original reservoir and initiate a successful chain of transmission in the new host (41). This perspective discusses each scenario and the factors that inform risk assessments.

HUMAN PATHOGENIC VIRUSES IN PERMAFROST

Smallpox

Smallpox is caused by the variola virus, a double-stranded DNA virus with a 186-kb genome (42). The last known natural case occurred in 1977, and the World Health Assembly declared it officially eradicated in 1980 (43). Since eradication, bodies from permafrost have been tested for evidence of viable virus at archeological sites or when warm temperatures threaten burial sites (15, 44). The first such test was in the 1990s, initiated by the discovery of a wooden vault, near the village of Pokhodsk in northern Siberia, that contained mummified bodies of smallpox victims (44). Scientists from the Vector Institute (a facility near Novosibirsk that is one of the two places on Earth that holds remaining stores of smallpox virus) and their colleagues collected samples from the chamber, including from smallpox pustules. Back in the lab, they were unable to isolate live virus from the samples (44–46) and explained that the virus may have been destroyed by prior freeze–thaw cycles since the burial site was near the permafrost surface and may have periodically thawed during warm years (44, 46).

Another attempt at testing for variola virus was from well-preserved frozen mummies at an archeological site in the central Sakha Republic in Eastern Siberia (15). Quick burial after death and preservation efforts during excavation and sampling increased the probability of virus preservation. Rather than attempting to recover viable virus, the length of variola virus DNA fragments was used as a proxy for the presence of intact virus. Since an intact genome is necessary for viability, infectivity would be possible only if long pieces of DNA remained. PCR products were obtained for three short DNA fragments (139, 145, and 590 bp), and sequence analysis confirmed amplicons were from the variola virus. Long-distance PCR (targeting an ~2-kb region of the viral genome) failed to produce amplification products, ruling out the presence of intact viral particles.

The influenza pandemic of 1918

The influenza pandemic of 1918, caused by an H1N1 influenza A virus with a 13.5-kb RNA genome, resulted in the deaths of tens of millions of people (47). The pandemic was particularly devastating for residents of Alaska Native communities, who suffered 82% of influenza-associated deaths in Alaska (48). The story of virus recovery efforts from human remains in permafrost is centered in Brevig Mission (called Teller Mission in 1918), a small Inupiat village. On 14 November 1918, Brevig Mission had ~80 adult residents. From 15 to 20 November, influenza claimed the lives of 72 residents. A burial site was dug into the permafrost, where the preserved remains of victims were undisturbed until 1951 when scientist Johan Hultin obtained permission from village elders to excavate the burial site (49, 50). Tissue was collected from the lungs of four individuals who had been interred in permafrost. To determine if infectious H1N1 virus persisted, the lung tissue was cultured in embryonated chicken eggs (51), a highly sensitive method for influenza virus detection (52). However, no evidence of infectious virus was observed (50, 51).

Forty-six years after the first attempt and 79 years after the pandemic, Hultin and colleagues again obtained permission to excavate the Brevig Mission gravesite for an attempt at H1N1 virus recovery. Lung tissue was collected from the remains of four individuals buried at a depth of approximately 2 m. Influenza virus RNA was detected in one of the individuals using reverse transcription PCR (RT-PCR) testing. However, only small fragments of the viral genome (<120 nucleotides) could be amplified (51, 53), showing that viable viral particles did not remain in the samples and suggesting that influenza virus does not survive in permafrost.

The interred remains of individuals from Brevig Mission represent the highest probability scenario investigated to date in terms of human pathogenic virus preservation. Gold miners, who were skilled at excavating in permafrost, were employed by the territorial government to dig the burial site. Individuals were buried at 2 m, below depths susceptible to occasional thaw during warm years. The 1951 excavation recovered "generous" biopsies from eight lungs, which all tested negative for the presence of influenza virus (51). In 1997, the remains of four individuals were uncovered (53). One was well preserved, which was likely due to subcutaneous fatty tissue that had protected the internal organs. It was tissue from that lung that yielded small viral genome fragments (51).

Lessons learned from variola virus and the 1918 H1N1 influenza virus

These examples suggest that human pathogenic viruses probably do not remain infectious when frozen in permafrost, even when preservation conditions are nearly ideal and samples are carefully collected using procedures to preserve viability or genome integrity (17, 54). The small number of examples makes the precise hazard difficult to predict, but also points to another consideration: specifically, that the number of well-preserved grave sites may be quite small. Digging into frozen ground is difficult and requires special tools and/or techniques (51). Burial in the active layer (soil overlaying permafrost that freezes and thaws annually), as occurred in the case of 1918 influenza victims on Svalbard (55), or in shallow permafrost that is near freezing and occasionally thaws during warm years, can reduce the number of viable viral particles by many fold due to freeze–thaw cycles (54). Even for recently deceased influenza victims, the infectious hazard of human remains is low. Guidelines for funeral directors and mortuary staff place influenza in the low-risk category, and viewing and embalming are considered safe (56–58). Together, the combination of limited infectious virus survival in permafrost, a potentially small number of well-preserved burial sites, and low transmission risk from human remains suggests that thawing permafrost is not a probable exposure pathway.

WILDLIFE AND DOMESTIC ANIMAL HOSTS

Reintroduction of a virus from thawing animal remains or water and soil that previously served as wildlife habitat could have potentially deleterious impacts on non-human animal populations. Human populations could be affected if the pathogen is able to shift hosts. There is, however, no precedent for a domestic animal or wildlife disease outbreak following emergence of a viral pathogen from frozen soils, nor recognized events where viruses from recently thawed permafrost caused zoonotic disease. This does not mean that such a scenario is impossible, but it does imply that it is improbable. The closest example, specifically the 2016 anthrax outbreak, was caused by an environmentally resistant endospore-forming bacterium rather than a virus, and it is still unclear whether thawing permafrost was a causative factor (22).

Pathogenic viruses in animal remains face the same degradative processes that compromise influenza and variola viruses in permafrost, as described in previous sections. Delays between death and entombment in permafrost, nucleic acid damage that accumulates over time, and exposure to freeze–thaw cycles cause the degradation of viral particles, limiting the potential for viable virus to persist in animal remains (59–62). Damage begins to occur almost immediately after death (59), causing a rapid decline in the number of infectious viral particles (63–65). A few exceptionally well-preserved

animal specimens show evidence of immediate burial in permafrost or ice (66, 67), but the majority exhibit signs of delay between death and the point of preservation (e.g., tissue destruction, partial decomposition, or dismemberment) (68–72).

Though permafrost slows nucleic acid degradation, significant damage still occurs in the form fragmentation, crosslinking, and lesion accumulation (62). DNA extracted from ancient faunal remains contains a mix DNA from the animal itself, microorganisms (including viruses) present at the time of death, and microorganisms introduced postmortem (59, 73, 74). This DNA is typically highly fragmented, usually less than a few hundred base pairs in length but often much smaller (73–77). RNA, when recoverable, is even more degraded (78). Even remains preserved soon after death experience nucleic acid damage (79, 80), which accumulates over time (59).

Most well-preserved animal specimens found in permafrost date back to the late Pleistocene (between 11,700 and 129,000 years ago) and early Holocene (between 8,200 and 11,700 years ago) (68, 81, 82). These timeframes exceed the expected survival of fully intact nucleic acids from viral and other microbial sources unless they are protected by stress-resistant forms (e.g., capsids that shield against harsh extracellular environments or cysts) or they have low levels of metabolic activity that enable DNA repair (79, 83, 84). Unlike viruses associated with microbial hosts in permafrost (discussed in the next section), animal viruses lack adaptations to the permafrost environment and do not have the protection of a cold-adapted host that can perform DNA repair or enter dormancy (11, 79, 83, 85, 86).

In stark contrast to the hypothetical risk posed by viruses from thawing permafrost, viruses circulating among wild and domestic animals have repeatedly jumped into human hosts, causing disease outbreaks. Examples include the viruses causing coronavirus disease 2019, Ebola, and avian influenza (87–89). This suggests that risks of disease outbreaks from viruses of animal origin are much more likely to stem from contact with extant wildlife (direct or indirect) in the Arctic and subarctic rather than emergence from permafrost (90, 91).

PERMAFROST VIRUSES WITH MICROBIAL HOSTS

Giant viruses in permafrost

In contrast to the viruses causing smallpox and influenza, numerous giant viruses have been revived from late Pleistocene-aged permafrost (31, 92). These giant viruses infect *Acanthamoeba* (31), a genus of amoeba that is broadly distributed in both natural and built environments (93, 94). Beyond permafrost, giant viruses have a widespread global distribution and can infect amoebae and other protist hosts such as algae (95–98). Their persistence in permafrost likely stems from a wide range of virus and host adaptations to extreme conditions (86). Giant virus capsids are incredibly stable (99), and their outer walls are comparable in thickness to Gram-positive bacterial cell walls (100), which enable the viruses to retain infectivity after exposure to chemicals, desiccation, and extremes in temperature, pH, and salinity (85, 101–103).

Beyond the robust viral structure, *Acanthamoeba* hosts may offer protection against extreme conditions (104). *Acanthamoeba* form rugged double-walled cysts (105–108) and may also have freeze-resistant non-encysted forms with low levels of metabolic activity (109). Encystment of an infected cell or maintenance of an infection in a non-encysted state could facilitate virus survival and enable reemergence when permafrost thaws.

Human pathogenic viruses are comparatively fragile and are not protected from extreme conditions by a cold-adapted host. Even variola virus, which can survive outside a host for several years in scabs and lesion crusts (often collected for variolation efforts) and is known for being stable (110), does not appear to survive for a few hundred years in permafrost (15, 54), let alone thousands of years as seen in giant viruses. Together, these data suggest that the persistence of giant viruses in permafrost is not a reliable indicator of the ability of human viral pathogens to likewise survive in permafrost.

Giant viruses have been associated with conditions in humans, such as pneumonia and keratitis, leading to questions about their potential to cause disease (111, 112). However, amoebae that host giant viruses are also present in such cases. These amoebae either directly cause disease (e.g., *Acanthamoeba* keratitis [113]) or play a crucial role in infections, such as facilitating the transmission of *Legionella pneumophila* in respiratory illness (114). There is no evidence of a causal link between giant viruses and disease (37). The absence of such connections strongly suggests that giant viruses are unable to infect humans directly. Associations between giant virues and humans are probably incidental, caused by viruses co-occurring with thier amoeba hosts (93, 99, 102, 107).

Other microbial viruses in permafrost

In addition to giant amoeba-infecting viruses, permafrost (like other soils) contains diverse viral communities with bacterial, archaeal, and other microeukaryotic hosts (9, 115–117). Stable isotope probing, RNA sequencing, and thaw experiments show that at least a subset of these viruses maintain viability and can be active at subzero temperatures (10, 118). In extreme environments, including permafrost, viruses can have multiple survival mechanisms, such as increased virion (viral particle) stability and integrity, auxiliary metabolic genes that aid host survival in cold conditions, and the ability to integrate their genetic material into the host genome, allowing the virus to coexist within a (cold adapted) host in a quiescent state (11, 12, 119–121).

Potential for viruses with microbial hosts to spillover to humans and animals

The theoretical risk posed by permafrost viruses with microbial hosts to human populations can be evaluated by exploring the origin and evolution of pathogenic viruses and examining whether there is precedent for microbial viruses spilling over to humans or animals. Host jumps between closely related species are common at evolutionary timescales; 61% of human pathogens and 75% of emerging human pathogens have zoonotic origins (122). The likelihood of successful host jumping decreases as the phylogenetic distance between hosts increases (123–125). Viruses bind specifically to molecules on the host cell surface, rely on the host's cellular machinery for replication, and must evade the host's immune system (126–128). As a result, they are typically highly adapted to specific hosts and can only "work" in hosts with similar molecular pathways (129, 130). Given the large phylogenetic distance between soil microbes and humans, such a shift would be extraordinarily unlikely, if not impossible.

Investigating the origins of human pathogenic viruses shows that microorganisms are not a source of spillovers. The majority of zoonotic viruses (>80%) are from mammals, while the remaining ~20% are primarily from birds (87, 124, 131, 132). Instances of viruses infecting both humans and other vertebrates, such as reptiles, are rare (131, 133, 134). Non-zoonotic viruses, such as papillomaviruses and herpesviruses, which are host specific, offer insights into potential host jumps in the more distant past. For these viruses, the host phylogenetic tree roughly mirrors the viral phylogenetic tree (135–137). For example, human papillomaviruses are most closely related to papillomaviruses with non-human primate hosts, and the phylogenetic distance between viruses increases with evolutionary distance between hosts (136, 138, 139). These evolutionary relationships can largely be attributed to co-speciation between virus and host and occasional instances of interspecies virus transfer (135, 137, 140). However, there is no evidence to support a microbial origin for these viruses (135, 137, 139, 141, 142).

The lack of precedent for microbial viruses jumping to human or animal hosts suggests this scenario is unlikely to arise from thawing permafrost. Humans are constantly exposed to an enormous diversity of viruses from the environment. Viruses are crucial members of all Earth's ecosystems (143), and soils are perhaps the largest viral reservoir on Earth (144). In some soils, viral abundance can exceed more than one billion per gram, and similar counts are found per gram of human intestinal content (144–146). Billions of viruses can be swallowed during a swim in the sea (147, 148). Despite this constant bombardment, there is no indication that microbe-infecting viruses

represent a major disease risk for humans, wildlife, or domestic animals (87). Research, surveillance, and prevention strategies aimed at mitigating the risk and impact of future pandemics do not identify these viruses as potential threats (90, 149, 150). There is no reason to think that viruses from permafrost represent a greater risk for spillover than viruses maintained in other environments such as temperate soils and aquatic systems.

CONCLUSIONS

Currently available data indicate that there is no increased risk of human viral pathogen emergence from permafrost compared to other environmental sources. We do not claim that viral pathogens in permafrost pose zero risk or that surveillance of putative viral pathogens in permafrost is unnecessary. However, there is currently no evidence that human or animal viral pathogens frozen in permafrost pose an imminent disease outbreak threat. Though climate change is accelerating thaw, the entry of microbes from ancient permafrost into the modern environment is not a new phenomenon. Permafrost is continually and naturally exposed to the modern environment by processes such as erosion, cryoturbation, frost heave, solifluction, wildfire, and climate fluctuations (151–153). Humans have been in the Arctic for more than 40,000 years (Alaska up to 25,000 years and Scandinavia ~5,000 to 12,000 years) (154–156). This suggests that people are and have been regularly exposed to viruses from permafrost soils, with no clear evidence for large-scale health consequences.

Framing viral discovery efforts in the cryosphere as a search for "zombie viruses" or potentially pandemic pathogens is not particularly useful and perhaps even harmful. Stoking fears of viruses in thawing permafrost may inadvertently discourage customary and traditional cultural practices among subarctic and Arctic residents or divert attention from the more pressing ways that pathogens in the warming Arctic pose risk to human health and well-being (157). For example, wildlife and humans increasingly occupy shared habitats, providing opportunities for viruses maintained in animals to spillover into humans (90). Arthropod and rodent disease vector ranges are expanding and shifting northward (158). Extreme precipitation and flooding events are increasing, threatening infrastructure such as water and waste treatment facilities (159–161). Higher temperatures increase the survival of some water-borne disease agents (91). Furthermore, wildlife health may be affected as a consequence of climate change, which further compromises fragile ecosystems and the people that rely upon them (162).

BOX 2. ZOMBIES ARE FICTIONAL AND HAVE RACIST ORIGINS

The frequent presentation of "zombie" pathogen in news articles and a few academic articles has received immense and improper attention (31, 163). The term zombie pathogen is typically shorthand for a pathogen from the past that has been preserved in permafrost and could become active and cause human disease when released by thaw. As we discuss here, this outcome is highly unlikely, and yet the term zombie virus is used to elicit an apocalyptic fear response in the reader, often to grab headlines. It is not well known, and thus important to examine, that the zombie fantasy has origins in the racist past against people of color, particularly in North America and the Caribbean (164, 165). It originated from the dehumanizing violence against Haitian slaves in the 17th century, born of Vodou folklore but then modified and used as a means of control and oppression (166). It was later made popular in horror movies containing escapist end-time fantasy (166, 167). Because of its racist origins, this mythology perpetuates the idea that "invaders" that are unlike us are coming to destroy our world and that destruction is imminent and fast moving (168). Despite the collective interest in zombies, it is important to note its cultural origin so that the concept can be more easily put aside as fiction, rather than a scientific metaphor. The word's use in scientific literature is unhelpful, provides only titillating headlines, and unconsciously supports a narrative of xenophobic fear of things we do not understand (169).

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