Invited Editorial/Commentary:

Title: Microbes and space travel - hope and hazards

Running title: Microbes and space travel

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The 2015 movie, 'The Martian', demonstrated how a lone astronaut, stranded on Mars, was able to survive by growing potatoes in Martian soil fertilized by human faeces, whilst awaiting a variety of rescue missions [1]. This is, of course, science fiction, but it illustrates the human fascination with the red planet. Recent, successful, real-life Mars exploration missions, such as the Perserverance/Ingenuity (USA) and Tianwen-1/Zhurong (China) are a further testimony to this. In addition, various privately funded ventures to travel to 'the edge of space' [2], demonstrate man's evolving technological capability to increase the accessibility of space exploration to the masses.

At the same time, due caution is needed, and this caution needs to be bidirectional - to protect other planets and moons from Earth's microbes (that can ride on interplanetary probes) that we *know* exist, but also to protect the Earth from potentially harmful extraterrestrial life-forms which *may* exist.

The spectacularly successful NASA (National Aeronautics and Space Administration) Galileo (to Jupiter, 1989-2003) and Cassini (to Saturn, 1997-2017) missions [3] demonstrated the possible presence of subsurface oceans of liquid water in several of Jupiter's and Saturn's moons [4,5]. Yet, at the end of these respective missions, both of these spacecrafts were deliberately destroyed by crashing them into Jupiter and Saturn. This was done to avoid contaminating any of their moons with possible organisms and debris from Earth [6]. In the reverse direction, potential sources of organic matter have been found in comets and asteroids, examples of which could have crashed into early Earth, seeding the development of life [7].

The famous Miller-Urey experiment [8] demonstrated the generation of essential amino acids in the presence of liquid water, with the input of electrical energy simulating lightning storms – conditions similar to those found on primordial Earth. More recently, Horst et al. [9] performed similar experiments, this time mimicking the conditions on Titan, where surface liquid water is absent, and reported the production of DNA/RNA bases.

Thus far, we believe that the generation of compounds that support life requires at least two essential conditions: a source of energy to drive chemical reactions, and liquid water allowing various organic molecules to mix and combine to form more complex structures to create life [10,11].

On planets distant from the Sun, like Jupiter and Saturn, their large gravitational forces can produce heat from volcanic and other geothermal activities on their orbiting moons, to supply the energy for the development and maintenance of life. For planets closer to the Sun, direct solar radiation can provide sufficient energy to do the same on planets orbiting within the so-called 'habitable zone' - at least, for carbon-based life 'as we know it' [10,11]. Indeed, the earliest life-forms on Earth may have started under the seafloor in low-temperature hydrothermal fluids, thriving in volcanic rock substrates, using methane instead of oxygen for metabolism [12]. This process may also occur in extraterrestrial settings.

Our first encounter with alien life-forms, therefore, will very likely be with primitive virus- and bacteria-like microbes which may have characteristics similar to terrestrial 'extremophile' organisms that can tolerate very high levels of temperature, pressure, acidity, alkalinity and salinity [13].

Life on Mars?

Mars is ~250 million km, and Earth is ~150 million km from the Sun, putting Mars just on the outskirts of our Sun's 'habitable zone', with a surface temperature of -63^{o}C, making liquid water impossible. The earlier 1976 Viking 1 and 2 Mars lander missions analysed Martian soil samples for the presence of life but found no conclusive evidence [14].

Similarly, when a meteorite fragment (ALH84001) thought to have originated from Mars was later recovered from Antarctica in 1984 and analysed for evidence of life, initial optimism over 'possible fossilised Martian bacteria' eventually faded when inorganic, alternative explanations seemed more likely [15]. Interestingly, the size of these 'fossils' ranged over 20-200 nm in size, which is compatible with some terrestrial viruses.

One notable feature of Martian soil was that it was found to contain high levels of perchlorates [16]. This suggests that any native Martian organism could be very tolerant of chlorine-based cleaning agents that are often used on Earth. Terrestrial microbes are generally very sensitive to chlorine which is a component in many commercial surface cleaning agents. This raises the interesting, practical issue that alternative chemicals will be required to 'disinfect' areas potentially contaminated by Martian microbes, if any are found. One innovative option would be the use of irradiation techniques for surface sterilization, such as high energy electromagnetic radiation (using gamma or X- rays) or high energy subatomic particles (e.g., electrons), in which the lethal effect on microbes in different materials, such as single-use personal protective equipment, has been well documented [17]**.**

Higher life-forms?

Although much less likely, possible encounters with higher life-forms deserve a brief mention, given the popularity of this concept in fiction and the media. The 'Search for Extraterrestrial Intelligence' (SETI: https://www.seti.org), and/or other manifestations of the various components of the famous Drake equation [18], are more likely to be found on exoplanets orbiting other star systems, rather than within our Solar System.

These are projects are attempting to look for signs of intelligent life capable of producing 'technosignatures', where life has evolved to a level that can produce signs of technical capability, such as atmospheric industrial pollution, orbiting megastructures, artificial radio signals, like those produced on Earth [19,20]**.**

Yet, on balance, we are much more likely to encounter extraterrestrial life as some form of microbe - similar to the bacteria and viruses that we see on Earth.

Extraterrestrial microbes?

The definition of life by NASA [21]**:**

"Life is a self-sustaining chemical system capable of Darwinian evolution"

implies an ability to replicate and adapt to its environment. To be capable of this requires some form of adaptive genetic, or other form of instruction code for life, which is directly or indirectly responsive to its environment.

On Earth we have DNA in bacteria, fungi and higher level organisms. We also have viruses that can infect all of the above that encode viral proteins using different forms of both DNA and RNA. The problem with viruses specifically is that they are inert and inactive until they find a suitable host cell, to which they can bind and enter for replication. Such terrestrial virus-host cell interactions are very specific - though some viruses like influenza can infect multiple hosts containing similar permissive cell types. Of course, this limitation may not necessarily apply to extraterrestrial viruses that attach and replicate in different ways.

Other forms of DNA or RNA may be found in extraterrestrial viruses. Stable synthetic DNA with 8 bases, including four new bases (P, B, Z, S) in addition to the usual four (A, T, G, C), has already been created here on Earth [22]. Given the variety of viral genomes that we see here on Earth [23], with viruses being able to infect virtually every other living organism, it seems eminently feasible that extraterrestrial viruses can exhibit a similarly wide spectrum of host tropisms - including the ability to transmit across multiple difference species.

Extraterrestrial viruses – mechanisms of disease?

Any mechanisms and manifestations of human infection from an extraterrestrial virus can only be speculative at this point, but is likely to fall within one of these categories, based on our wide experience with terrestrial viruses:

Transmission: For terrestrial viruses this can occur via several different routes, including combinations of: *direct contact* with human mucus membranes (eyes, nose, mouth), including via kissing and sex (e.g. herpesviruses, HIV, HBV); blood transfusion or organ transplantation, and other *bloodborne* modes of transmission, as exemplified with intravenous drug use (e.g. HIV, HBV and HCV); *vertical transmission* (from mother-to-child, e.g. VZV, CMV, rubella, parvovirus B19, Zika virus); *large droplet* spray landing directly on mucous membranes, or *aerosols* where airborne virus in smaller liquid particles are inhaled (e.g. respiratory viruses, enteroviruses, parvoviruses, measles, mumps, rubella) [24,25]. One alternative is that these viruses may be able adapt to other terrestrial life forms like insects, birds, mammals (including bats), plants, fungi, bacteria, etc. initially, before then adapting to and infecting man as a zoonotic or *vectorborne* infection, as we have seen with HIV, Ebola, SARS-CoV-1, MERS-CoV, avian influenza A(H5N1), A(H7N9), and SARS-CoV-2.

Pathogenesis: Once inside a human host, the alien virus needs to find a *permissive cell* that allows it to enter to replicate further. Assuming that such viruses have not encountered humans before, there would need to be some degree of coincident *molecular mimicry* - to 'mimic' some terrestrial virus' receptor binding sites/domains - to enable *virus entry* into human cells expressing surface receptors that look similar to cells from the alien virus' normal host. Unless this specific molecular mimicry exists in such alien viruses, humans may be protected from infection. However, if alien viruses exist in multiple forms, it might be possible

that at least one strain could mimic human viruses closely enough to be able to bind and enter a human cell, whilst simultaneously *evading* human host innate immune responses.

Presentation: Viral infection could then lead to several typical *clinical manifestations*: a *febrile prodrome* with some form of rash, like measles, rubella, parvovirus B19, VZV (chickenpox), dengue; a *gastroenteritis* and diarrhoea, like norovirus, rotavirus, enteric adenovirus; a *respiratory* illness, like human and avian influenza, Sin Nombre hantavirus, SARS-CoV-1/SARS-CoV-2; or remain 'silent' and *asymptomatic*, yet still infectious, like HIV, HBV and HCV; a chronic infection with longer-term *oncological* illness, like HTLV and HPV; an acute and/or longer-term *neurological* problems like West Nile, Japanese or Nipah encephalitis; an *acute flaccid paralysis* like polio or enterovirus D68; more *specific syndromes* like hantavirus HFRS (haemorrhagic fever with renal syndrome); severe fever with thrombocytopenia syndrome (SFTS)**,** caused by a bunyavirus; any *combination* of these presentations as part of a more *systemic* illness, like those caused by measles, VZV, adenoviruses, enteroviruses, SARS-CoV-2, Ebola and other viral haemorrhagic fevers, smallpox [24,25].

Treatment and Control: Clearly, initially, there will be no specific *antiviral* treatment or *vaccines* for such alien viruses, though existing drugs can be repurposed in an attempt to treat the infection, as we've seen with SARS-CoV-2. Uncertainties with the mode of transmission may lead to confusion and ineffective interventions - again, as we have seen with SARS-CoV-2 - which may also lead to high levels of infection, morbidity and mortality, as we try to find ways to combat this virus, as it spreads through the global human population.

Specific impact of radiation and microgravity on host-pathogen relationships

There are concerns about how micro- (or zero) gravity may impact on bacteria onboard orbital space vehicles and space stations. These concerns become more relevant as space travel and longer durations of stay become increasingly accessible with multiple private enterprises.

Under both simulated microgravity environment as well as on board spaceflights and the International Space Station, bacteria have been shown to undergo novel mutations to adapt to this unique environment [26,27]. Some of these adaptations to space flight alter the pathogen life cycle, some confer tolerance to harsh environments (e.g. osmotic, acid and alcohol stresses), and others have a variable effect on virulence – including, decreased doubling time due to a shorter lag time, increased time in the exponential phase, resulting in a high final cell density compared to ground controls (*Escherichia coli* K-12); increased virulence in a mouse model, yet decreased virulence in a *C. elegans* model for *Salmonella typhimurium*; increased biofilm mass and thickness, but unchanged morphology (*Pseudomonas aeruginosa*) [26].

These variable adaptations are likely to be dependent on environmental conditions, as well as specific bacterial strains [26,27]. Some of these adaptations may become more permanent with time, thus raising the possibility of their inadvertent introduction into the wider bacterial flora on Earth upon return [27,28]. Such introductions may lead to more severe infections in individual patients, but also larger, more severe outbreaks in the population of

these ‘space-enhanced’ pathogens [27]. Yet other studies suggest that there is currently no clear, consistent or definitive evidence that these adaptations pose actual risks to the health of space travellers either inflight or upon their return to Earth [28,29].

From the viewpoint of the host (humans), it is known that the immune systems of astronauts can become weakened by prolonged exposure to zero or microgravity [27,30]. This *host immunosuppressive* effect of microgravity also allows the *reactivation* (a fairly reliable clinical sign of host immunosuppression) of some latent terrestrial viruses, like herpesviruses (HSV, VZV, EBV, CMV), resulting in increased viral shedding and specific skin lesions, such as cold sores (HSV) and shingles (VZV) [30]. Thus, the microgravity environment could also enhance the potential *virulence* (clinical disease severity) of any extraterrestrial pathogens.

Another environmental factor that could have an impact on microbes, and how they evolve, is the effect of radiation (ionizing and non-ionizing). The relative distance from and the specific properties of a planet’s star will determine the dose and type of radiation to which it is continuously exposed, e.g., the dose of ultra-violet (UV) light or the induced flux of high-energy protons. Another source of planetary radiation comes from so-called ‘cosmic rays’, which are high-energy, fast-moving (near the speed of light) subatomic particles that collide with the upper atmosphere of planets and moons. These collisions produce a spray of secondary particles that enter the planetary environment, and the exact nature of these particles will depend on the characteristics of the atmosphere. The outcome and nature of these interactions will determine the level of the planetary background radiation, which in turn will impact on how life develops there.

On Earth, radiation induced by cosmic rays at sea-level corresponds to about 13% of the total background annual effective dose in the population (from natural sources), although for higher altitude cities this factor increases to about 30% [31]. As for UV light, a specific, short wavelength range, typically from 100–280 nm (UV-C), has enhanced sterilization properties, though natural UV-C is blocked from reaching Earth’s surface by the ozone layer. Even though artificial UV-C light is used for air sterilization, the other two longer wavelength categories of UV spectrum (UV-A: 315-400 nm and UV-B: 280-315 nm) also play an important role in Darwinian evolution.

For example, whilst sunlight is essential for phytoplankton that reside in water to perform photosynthesis, UV-A and UV-B radiation inhibit photosynthetic production, thus reducing the generation of oxygen and reducing the consumption of carbon dioxide by these microorganisms. In a balanced environment, just by increasing or decreasing the dose of UV-A or UV–B radiation, this would alter the photosynthetic production, which can positively or negatively affect the planet’s carbon cycle.

In other extraterrestrial environments, the natural contribution of UV- A, B or C, or the background cosmic-induced radiation could be lower or higher, inducing a direct effect on the viability of specific types of microbes and their capacity to adapt to that particular environment.

Future Perspective

With plans to return Martian soil and rocks to Earth in the near future for examination, the possible presence of extraterrestrial life-forms in these samples is both a hope and a hazard.

Careful and meticulous precautions should be developed and applied to avoid their contamination with terrestrial organisms, but also the escape of any Martian life-forms into Earth's environment. At the same time, continued surveillance for any micro (and zero)-gravity-induced bacterial mutations should be initiated and maintained during extended flights or orbits, including in those undertaken by private enterprises, where adequate biosafety and isolation-quarantine protocols may not be well-developed.

Mankind's history is littered with various plagues [32]. Even with newer pathogen detection and surveillance technologies available, as aptly demonstrated in the current SARS-CoV-2 pandemic in many Western countries, we need to be better prepared and quicker to react, if we are to stop any novel infectious agent from spreading globally - both terrestrial and potentially, extraterrestrial.

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