

PEARLS

They are what you eat: Shaping of viral populations through nutrition and consequences for virulence

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Introduction

Humans have coexisted with viral pathogens for tens of thousands of years, influencing both their emergence and evolution. However, the pervasiveness of the Western diet and disparities in food access and security have altered how we as hosts interact with our viral pathogens. Malnutrition, the state of having insufficient, excess, or imbalanced sources of energy, is well known to attenuate immune responses. Could nutrition also actively shape how viruses evolve? Malnourishment is a global, intersectional issue, and it may soon force a revision of our understanding of how viruses evolve within their hosts ([Fig 1](#)) [1].

Why do RNA viruses form quasispecies?

First theorized over 4 decades ago [2], a quasispecies population structure has been documented in plant, animal, and human pathogens [3–5]. A viral quasispecies describes the mutant but related genomes that collectively infect, replicate, and spread among hosts. Traditionally, the theory has been applied to RNA viruses. Because of their short generation times, small genomes, and the inherent lack of proofreading in most RNA replication, single nucleotide variants (SNVs) emerge at a rate of roughly 10^3 to 10^7 more mutations per nucleotide copied compared with DNA viruses [6].

Nonsynonymous SNVs are continuously accrued and purged from the viral genome. This flux generates a related “swarm” of viruses, which have little effect on the consensus sequence but may show phenotypic differences. Mutations with phenotypic consequences are generally deleterious; very few mutations have any fitness benefit. However, if beneficial mutations arise, they may relate to host range, drug resistance or vaccine escape, and replicative capacity [7, 8]. Both beneficial and the common deleterious mutations balance the structure of the viral swarm through complementation, interference, and cooperation [9–11]. Within a single host, tissue-specific subpopulations may vary in virulence without affecting consensus sequence or phenotype [12, 13]. Importantly, the consensus sequence should not be considered the “fittest sequence,” because selection, competition, and genetic drift act upon the entire viral swarm. Therefore, fitness of the swarm exceeds clonal sequence fitness, highlighted by work in vesicular stomatitis virus [3] and bacteriophage systems [14].

Viruses are obligate intracellular parasites that require a host cell to complete their life cycle. Barriers to replication exist within and between susceptible hosts, which restrict viral population diversity to quell infections [13]. In these wide-ranging environments, a

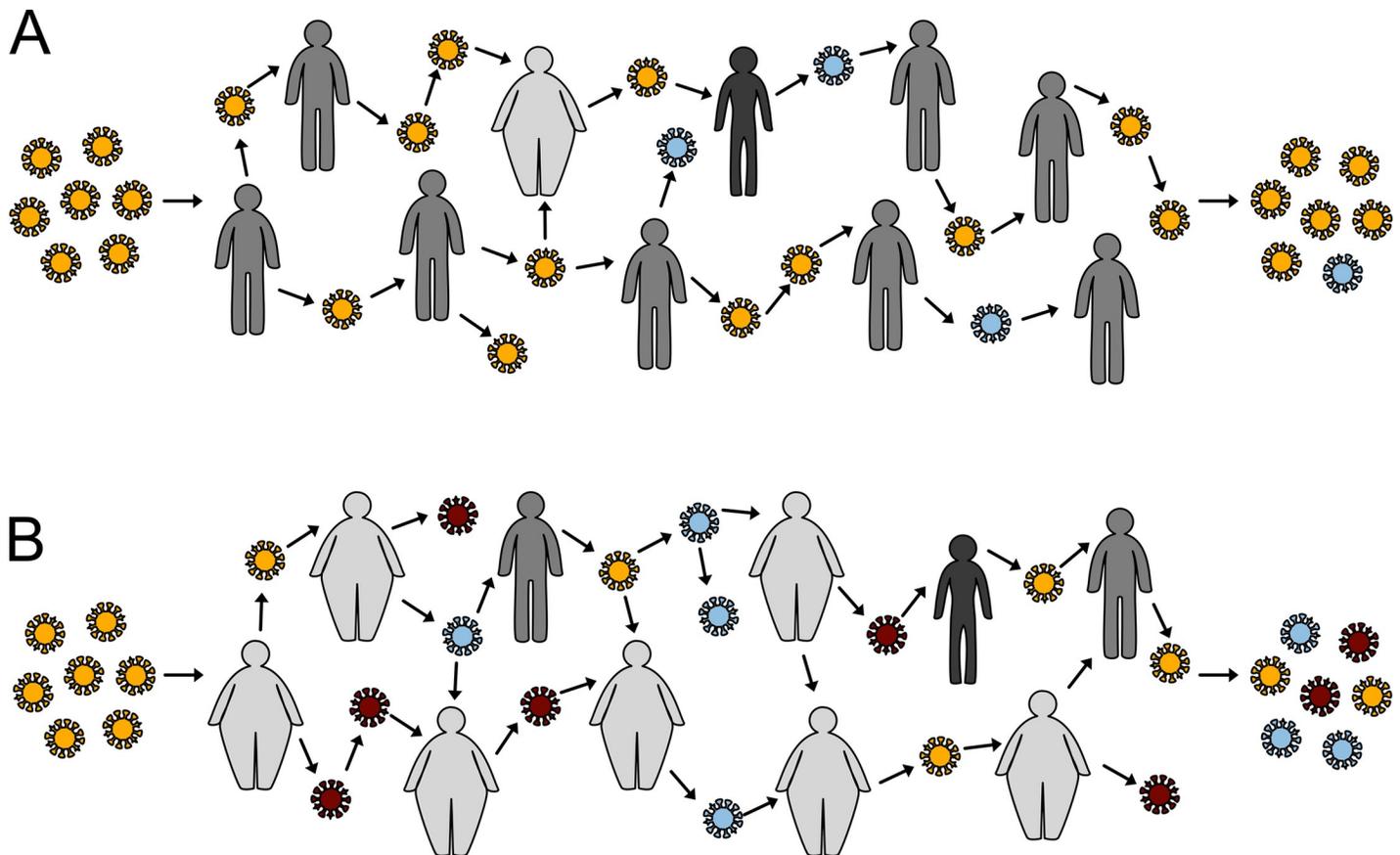


Fig 1. Prevalence of malnutrition may impact global viral evolutionary patterns. Worldwide rates of obesity are projected to reach over 50% by 2050. Additionally, undernutrition of both children and adults continues to be a global health crisis. (A) In a primarily healthy, lean environment, viral spread and acquisition of variants is limited as a result of robust immune responses. (B) As the incidence of malnourishment has increased, we have discovered that the resulting diminished immunity can permit the acquisition of minor variants and a virulent phenotype. In our current environment of widespread obesity, global viral evolutionary patterns may change because of increased host susceptibility and decreased host immunity.

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heterogenous viral swarm containing isolates with differing abilities to infect, transmit, and survive environmental and immunological onslaughts may safeguard viral existence. However, this genetic plasticity has bounds, with an evolutionarily beneficial middle ground between high- and low-fidelity replication [15, 16]. The “Goldilocks” approach maximizes fitness by avoiding lethal mutagenesis while ensuring amenability to selective pressures [17]. Too low fidelity leads to error catastrophe and collapse of the viral population; conversely, a highly clonal population may be extinguished by host defenses [18–21].

What is the implication of viral diversity on disease severity?

Numerous theories have questioned the biological relevance of a quasispecies and challenged its significance [17, 22]. However, boosting genetic diversity—to a point—is theorized to increase virulence. A viral swarm may be better equipped to face bottlenecks imposed by infecting hosts, environmental persistence, and transmission. Even within a single host, blockades due to infection barriers and the immune response diminish sequence variation, leaving a relatively homogenous population until replicative errors replenish the mutant pool [13]. So, do viruses harboring higher genetic diversity initially fare better in establishing an infection and displaying virulent phenotypes?

In studies with classical swine fever virus, higher genetic diversity correlated with virulence [23]; however, this conclusion has been challenged [24]. In other animal viruses, diversity increases precede the selection of virulent genomes [4]. Parallel conclusions have been made for human pathogens. In hepatitis C virus (HCV)-positive patients, high viral diversity prior to transplantation correlated with higher liver fibrotic scoring 1 year post-transplantation [5]. Continued genetic evolution of HCV correlated with progressing hepatitis, whereas resolution was associated with genetic stasis of HCV population [25, 26]. A model low-fidelity RNA-dependent RNA polymerase (RdRp) poliovirus variant demonstrates that increasing genetic diversity may not always yield fit populations [10, 27], yet high-fidelity RdRp mutants producing nearly clonal populations display reduced fitness *in vivo* [21].

Do host characteristics influence quasispecies structure?

Selection pressures ranging from host antiviral responses to pharmaceutical interventions mold the viral swarm. Upon infection, immune responses restrict genetic diversity by limiting spread and replication, eloquently demonstrated using a model poliovirus RdRp [13, 20]. Host immunological status is implicated in molding the quasispecies of dengue virus [28], norovirus [29], influenza virus [30], and coronavirus [31], among others. From these findings, empirical studies have found that host features responsible for attenuating immunity are also implicated in shaping the quasispecies and virulence, including aging [32] and immunocompromised status [29, 30, 33, 34].

Exogenous control of infections can affect viral swarm composition. As hosts, we have exploited the high mutation rates of viruses by redirecting viral evolution toward error catastrophe via pharmaceutical interventions [18, 19]. Interestingly, high-fidelity foot-and-mouth disease viral variants possess a higher level of resistance to pharmacologics but are attenuated *in vivo*, suggesting that the resulting restricted quasispecies hampers adaptability in the presence of drug or host pressures [19]. Also, antiviral treatment can lead genetic diversity gains that may precede selection of drug-resistant genotypes, as has been observed with oseltamivir [33, 35].

Is there evidence for altered viral evolution in malnourished hosts?

Globally, 1 in 9 people are undernourished and 1 in 3 are overweight or obese, with innumerable others suffering from micronutrient deficiencies [1]. Consequently, it is of utmost importance to understand whether host nutrition actively shapes how viruses evolve because many hosts do not mirror the actively studied “wild-type” condition. Previous work has identified micronutrient deficiencies that may increase pathogen virulence through acquisition of minor variants. In mineral- and vitamin-deficient mice, genetic mutations arise in coxsackie B and influenza virus populations that promote virulence even in well-nourished hosts [36–40].

In our work with influenza virus, we determined that nutrient excesses can drive virulence through population diversification [41]. Experimental evolution of CA/09 virus through two models of murine obesity resulted in a viral population displaying increased virulence upon inoculation of a wild-type host. This phenotype was not strain specific; an avirulent H3N2 virus was, upon passage in obese hosts, able to productively infect immunocompetent mice. We observed a significant increase in viral diversity and subsequent virulence after a single round of infection, with the phenotype persisting in obese-derived viral populations across 10 passages [41]. Interestingly, arbovirus-infected obese or protein-deficient mice showed higher morbidity but lower viral diversity, and both malnourished models transmitted virus less efficiently, highlighting that the effects of nutrition may vary based on the natural life cycles of

viral families [42]. It is yet to be determined how malnourishment may impact transmission of a respiratory, as compared with a vector-borne, virus.

How could what we eat shape our viral pathogens?

Both undernourishment and obesity are two sides of the same coin and are implicated in blunting immune responses and increasing susceptibility to infection [43, 44]. In our studies with influenza virus, we linked the emergence of a more diverse and virulent viral population with blunted interferon responses in obese hosts. Interferon treatment of obese mice restricted the emergence of a diverse quasispecies and attenuated the virulence of the resulting viral population, strengthening the claim that a robust innate immune response restricts subsequent infection severity, possibly through reduced viral replication and acquisition of a genetically diverse viral population [8, 20, 41]. Dietary metabolites also influence cellular metabolism and can push the body to a state of meta-inflammation; this prooxidant environment may also directly influence the genetic composition of the viral population [45].

Nutritional excess or deficiency may dampen the host immune responses and alter cellular metabolism, indirectly fostering an advantageous environment for viruses to explore the sequence space (Fig 2). The dearth of host responses to infection—particularly innate immunity—and the baseline malnourished state facilitates greater viral replication, permits the diversification of the viral swarm, and potentially allows for the emergence of advantageous mutations. Other indirect consequences of poor nutrition may also be involved. Blunting of immune responses may alter viral tropism and viral- or immune-induced pathology, thus remodeling the microenvironment in which the virus attacks the host. Also, nutrition is increasingly appreciated as an influence on the gut microbiome (reviewed in [46]). Interestingly, perturbations to the microbiome—both respiratory and gut—dampen interferon responses to respiratory virus infection [47–49]. However, to our knowledge, no empirical studies connect the obese microbiome to modulating enteric or respiratory viral populations.

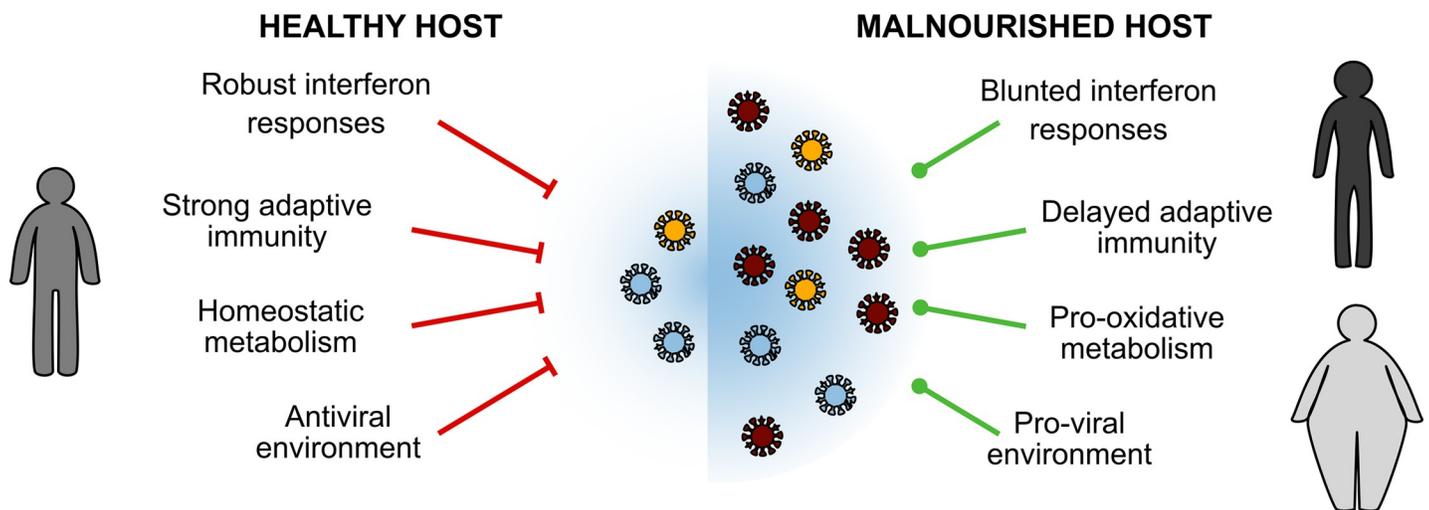


Fig 2. Direct effects of malnutrition on host antiviral responses shape the viral population. Nutritional status can alter the baseline state of host cells and the antiviral response postinfection. These departures to healthy metabolism and robust immunity may cultivate a proviral environment for increased replication and perhaps permit acquisition of beneficial mutations due to reduced selective pressures on the viral population. Other indirect effects of malnutrition could also alter viral interactions with resident microbiota. Finally, the ripple effects of over- and undernutrition are systemic for the host; however, nutrition status may have differing impacts for the virus based on tissue tropism, route of transmission, and mode of pathology.

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Conclusion

Pathogen virulence is a complex interplay of both host and pathogen properties. Host nutritional status has long been considered a risk for infection susceptibility and severity and is now implicated in shaping viral evolution. Continued studies on the molecular consequences of obesity and malnutrition at the macro- and micronutrient levels will reveal which host defenses are impaired through malnutrition and how they control quasispecies development and viral pathogenesis. Similarly, as we gain insight into how hosts influence quasispecies formation and pathogen virulence, we too can exploit these features for host benefit [18, 50].

The global ubiquity of malnutrition is shifting our population toward a more susceptible state. This will undoubtedly influence how pathogens behave within and between hosts. Continued study of how quasispecies evolution relates to other human, animal, and plant pathogens will indeed usher in a greater understanding of host–pathogen interactions and provide novel insights into how pathogens impact hosts and hosts impact pathogens.

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