The Three Ts of Pathogen Evolution During Zoonotic Emergence

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Abstract

When novel zoonotic diseases like Sars-CoV-2 emerge, they are likely to be poorly adapted to humans. Effective control measures will suppress transmission before significant evolution can occur, but extended transmission in human populations allows time for selection pressures to act. In this review, we discuss the factors shaping zoonotic pathogens' transmissibility and virulence at spillover and the selection pressures acting on these traits during emergence into human populations. We discuss how selection pressures during epidemics of emerging zoonotic disease are determined by the three Ts: trade-offs, transmission, and time scales. In short, virulence and transmission may trade-off, but transmission is likely to be favored by selection early in emergence. However, the relative selection pressures on transmission and virulence shift depending on the time scale of the epidemic. Predicting pathogen evolution in zoonoses therefore depends critically on understanding both the trade-offs of transmission-improving mutations and the time scales of selection.

Introduction

The current pandemic has emphasized that zoonotic emerging infectious diseases are undeniably a grave public health concern (Woolhouse et al., 2012). Clearly, these diseases necessitate rapid research upon emergence to uncover the pathogen's biology and modes of transmission in order to develop diagnostics, public health recommendations, treatments, and vaccines (Holmes et al., 2018). If successful, these interventions can stop transmission chains and end the epidemic. If these interventions are unsuccessful, however, extended circulation in humans can create selective pressures on these zoonotic pathogens (Plowright et al., 2017). Therefore, in extended outbreaks, some attention should turn towards monitoring and understanding potential pathogen evolution. Robust public health surveillance systems that include viral sequencing can identify potential adaptive variants (Korber et al., 2020; Rambaut et al., 2020) and evolutionary theory can help us understand how host, pathogen, and ecological traits shape selective pressures to determine possible evolutionary outcomes (Bonneaud & Longdon, 2020; Day et al., 2020).

Many recently emerged zoonotic pathogens have been viruses, particularly RNA viruses, whose high mutation rates mean that multiple variants reach high frequencies early in epidemics (Geoghegan et al., 2016). Most mutations in viruses have deleterious or neutral fitness effects (Sanjuán, 2010), but the small proportion of mutations with beneficial fitness effects might be particularly important for emerging zoonotic pathogens adapting to human hosts (Parrish et al., 2008; Plowright et al., 2017). However, even when these beneficial mutations occur early in the epidemic, they are slow to spread because selection pressures are weak relative to stochastic factors like drift in small populations (MacLean et al., 2020). Potential adaptive variants can also be difficult to identify because phylogenetic patterns are often complicated by human demographic factors and founder effects (Villabona-Arenas et al., 2020). For example, a variant of Ebola virus in the 2016 epidemic seemed to be associated with increased human

67 transmissibility in phylodynamic and *in vitro* assays, but did not correlate with higher viral titers or
68 shedding in macaques (Diehl et al., 2016; Marzi et al., 2018; Urbanowicz et al., 2016).
69 Despite these challenges, stories about mutations often spark public concern about
70 pathogens evolving to be more deadly, more transmissible, or to evade vaccines and treatments
71 (Grubaugh, Petrone, et al., 2020). Alternatively, historical theories of evolution towards avirulence
72 still pervade the public consciousness and sometimes lead to the prediction that the virus will
73 quickly evolve to become less dangerous (Smith, 1904). During the current Sars-CoV-2 epidemic,
74 reports of a mutational variant (D614G) increasing in frequency set off these debates in May
75 (Korber et al., 2020). Early responses cautioned against the overinterpretation of these reports
76 (Grubaugh, Hanage, et al., 2020; Grubaugh, Petrone, et al., 2020; MacLean et al., 2020;
77 Villabona-Arenas et al., 2020), but recent experiments in human cell culture and *in vivo* rodent
78 models have confirmed that this D614G variant may improve human transmission through higher
79 infectivity and replication in upper respiratory tissues (Hou et al., 2020; Plante et al., 2020). More
80 recently, the B.1.1.7 lineage with multiple spike mutations emerged in the UK and seems likely to
81 increase transmission rate (Rambaut et al., 2020). Despite these increases in transmission, the
82 D614G variant does not seem to be associated with changes in clinical severity (Volz et al., 2020)
83 and primary reports suggest that the B.1.1.7 lineage may not cause increased mortality either
84 (Davies et al., 2020). What is clear is that these examples show the potential for evolutionary
85 change during disease emergence.
86 Given the plausibility of Sars-CoV-2 adapting to improve human transmission and public
87 fascination with the topic, it is important that the broad scientific community have a clear
88 understanding of virulence evolution theory to quickly combat any false narratives. The study of
89 virulence and transmission evolution in epidemics of emerging infectious disease has been an
90 active but often separate area of research in both evolutionary virology and eco-evolutionary
91 theory (Cressler et al., 2016; Geoghegan & Holmes, 2018). In this review, we will integrate insights

92 from disease ecology, virology, computational genomics, and population genetics and eco-
93 evolutionary theory to form a more complete understanding of the factors shaping pathogen
94 evolution (Visher & Boots, 2020). We will discuss: how a pathogen's evolutionarily stable (long
95 term 'optimal') strategy depends on trade-off shape; what predicts pathogen virulence at the
96 spillover barrier; why selection pressures favor transmission improvements in maladapted
97 zoonotic pathogens; and how these selection pressures change over time during epidemics.
98 Through this, we describe predictions for pathogen evolution during epidemics of emerging
99 zoonotic disease and how they change depending on pathogen factors and host population
100 structure.

101

102 **Introduction to the Three Ts: Trade-offs, Transmission, and Time Scales**

103 The adaptive evolution of any trait depends on the presence of variation and the ability of
104 selection pressures to act on that variation. It is clear that pathogens, particularly RNA viruses,
105 can quickly generate and maintain large amounts of variation (Geoghegan & Holmes, 2017).
106 Selection pressures on these variants are weak compared to stochastic and demographic
107 pressures at the start of an epidemic, but gain strength as the number of infections increase
108 (MacLean et al., 2020). An extensive body of literature suggests that selection pressures on
109 virulence during epidemics of emerging zoonotic disease are determined by the three Ts: trade-
110 offs, transmission, and time scales (Anderson & May, 1982; Bull & Ebert, 2008; Day et al., 2020;
111 Lenski & May, 1994). See Figure 1 for graphical summary.

112 Theory has often assumed, and empirical data has increasingly shown us, that many
113 pathogen traits, like transmission and virulence, **trade-off** with each other (Acevedo et al., 2019;
114 Anderson & May, 1982; Cressler et al., 2016; Frank, 1996; Table 1). The **trade-off** theory is
115 important because it explains how different intermediate virulence, transmission, and recovery

rates can be optimal for a pathogen due to constraints between these key traits (Anderson & May, 1982; Cressler et al., 2016; Frank, 1996). It is often assumed that these trade-offs arise because these traits all correlate with within-host pathogen replication rates, although this is not necessary if symptoms directly correlate with transmission (Bonneaud et al., 2020). A large body of eco-evolutionary theory has shown that the shapes of these trade-offs determine the pathogen's optimum strategy and thus the direction of selection pressures (Anderson & May, 1982; Frank, 1996).

Emerging zoonotic pathogens typically do not have histories of selection in human populations and thus are likely to be maladapted for human-to-human transmission (Warren & Sawyer, 2019; Woolhouse et al., 2005). Meta-analyses of field data on recently emerged pathogens can tell us patterns associated with a novel zoonotic pathogen's ability to transmit in humans (Geoghegan et al., 2016; Guth et al., 2019; Olival et al., 2017). This can tell us about the extent of maladaptation to humans and establishes a starting point for selection. In theory, this maladaptation means that emerging zoonotic pathogens may initially have 'no-cost' mutations available that improve transmission without impacting traits like virulence (Bull & Ebert, 2008). In these cases, selection pressures for **transmission** improvements are likely to be most important (Bull & Ebert, 2008).

Finally, transmission increases continue to be the most important selection pressure on pathogens at the start of an epidemic, even when they trade-off with virulence (Lenski & May, 1994). Theory combining population genetics and eco-evolutionary approaches has shown how the relative selection pressures on different pathogen traits shift as the density of infected and susceptible hosts changes during an epidemic (Day & Proulx, 2004; Lenski & May, 1994). Therefore, the pathogen's optimum strategy changes over **time** during an epidemic. Preliminary evolutionary epidemiology modelling of Sars-CoV-2 has shown that evolution can vary depending

on these three Ts: trade-offs, transmission, and time scales (Day et al., 2020). We will describe each of these in detail below.

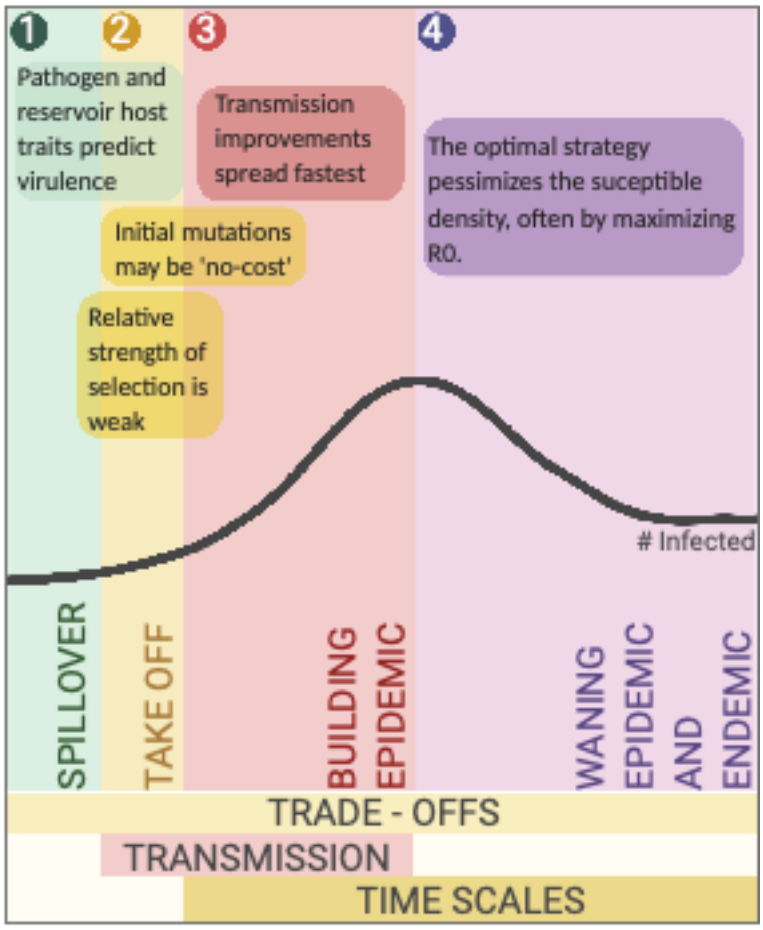

Figure 1: The Three Ts of Pathogen Evolution During Zoonotic Emergence

The Virulence-Transmission Trade-Off Hypothesis

Evolutionary biologists have long been entranced by the question of why pathogens harm their hosts, or cause virulence (See Box 1) (Fenner & Ratcliffe, 1965). Based on the assumption that host damage was always detrimental to parasite fitness, early ideas predicted that all parasites should evolve towards avirulence (Alizon et al., 2009; Smith, 1904). This was considered the 'conventional wisdom' until the 1980s, when foundational papers began to appreciate that virulence might be linked to other parasite traits like transmission or recovery rate

and therefore could have an evolutionary optimum (Anderson & May, 1982; Ewald, 1983). Any trade-offs between these traits would mean that low virulence could come at a cost of low transmission or fast recovery and that avirulence would therefore hinder parasite fitness. This virulence and transmission trade-off is now fundamental to our theories on pathogen evolution.

Theory on the virulence and transmission trade-off typically suggests that virulence and transmission are both functions of the within-host exploitation or replication rate (Alizon et al., 2009; Cressler et al., 2016). Because faster replicating pathogens generate larger population sizes, they increase their transmission rate while causing more host damage (Cressler et al., 2016; Frank, 1996). This damage increases host mortality, thereby decreasing the host's infectious period and providing a shorter window for the infected host to contact susceptible hosts (Anderson & May, 1982). In short, faster within-host replication increases the likelihood of infection upon contact while decreasing the overall duration of infection (Anderson & May, 1982; Frank, 1996). Under the trade-off hypothesis, parasites are therefore selected for exploitation rates that balance virulence and transmission (Anderson & May, 1982; Cressler et al., 2016; Frank, 1996).

Several other trade-offs have been proposed that don't depend on virulence and transmission trading off through the within-host exploitation rate. A virulence-recovery trade-off can occur if low replication rates make pathogens easier to clear such that lower virulence trades off with faster recovery rates (Anderson & May, 1982). Alternatively, a transmission-recovery trade-off can happen if the immune response is activated in a density dependent manner so that high replication rates have high transmission, but fast recovery (Alizon, 2008). A sickness behavior-transmission trade-off may happen if faster replication rates make the host feel sick and isolate themselves so that high replication leads to higher transmission rates, but fewer contacts (Ewald, 1994). Finally, the virulence and transmission trade-off does not necessarily depend on changes to the within-host replication rate if symptoms themselves are needed for transmission

(Bonneaud et al., 2020). These alternative trade-offs can all still lead to selection for parasites to balance their virulence or transmission metrics with other traits.

In simple host-parasite models, pathogens are selected to maximize the epidemiological R_0 (i.e. the number of secondary infections that a parasite produces during its infectious period in an entirely susceptible population) (See Box 2) (Anderson & May, 1982; but see Lion & Metz, 2018). The virulence-transmission trade-off predicts that these two traits are positively correlated, but the shape of this relationship is critical to the predictions of evolutionary theory (Anderson & May, 1982; Frank, 1996). When the trade-off is linear, pathogens evolve maximum virulence; but when the trade-off is saturating (such that virulence is acceleratingly costly in terms of transmission), pathogens will evolve towards an intermediate virulence (Alizon et al., 2009; Anderson & May, 1982). Given the centrality of the trade-off hypothesis to our understanding of virulence, it is noticeable that there are an increasing number of empirical studies that have found support for the core idea (See Table 1) (Acevedo et al., 2019).

While virulence evolution has traditionally been discussed in terms of R_0 maximization, R_0 does not directly correlate with pathogen fitness. A more universal rule is that parasites are selected following a pessimisation principle where the evolutionarily stable strategy is that which can be sustained in the lowest quality environment (Lion & Metz, 2018; Metz et al., 2008; Mylius & Diekmann, 1995). In virulence and transmission trade-off models, this is the strategy with the lowest susceptible population at ecological equilibrium. Under the pessimisation principle, the key insights of the trade-off hypothesis still hold in more varied, complex ecological circumstances (Lion & Metz, 2018). If virulence trades off with other parasite fitness components, selection will balance the negative fitness contributions of virulence with the positive fitness contributions of traits like transmission (Lion & Metz, 2018).

Box 1. Defining virulence

A textbook definition of virulence is "Whereas 'pathogenicity' refers to the capacity of micro-organisms to cause disease, the essentially synonymous term virulence is generally used to note variations in degree. Virulence encompasses two features of an organism's disease-producing capacity: infectivity (i.e., the ability to colonize and invade a host) and severity of the disease that is produced" (Davis et al., 1990; Read, 1994). Different subfields, however, emphasize different parts of this definition with plant pathologists focusing more on infectivity and animal disease focusing more on severity. In the context of the virulence and transmission trade-off theory, virulence is defined more narrowly as the additional rate of mortality due to infection (Read, 1994). In these models, virulence is therefore a host outcome that is mediated by host, pathogen, and environmental traits. Host and pathogen traits involved in virulence are similar in human and other animal systems and include traits like host age and genetics and pathogen replication rate and immune manipulation. Environmental factors involved in causing virulence for humans include resource availability (including access to healthcare), exposure to environmental toxicants, and environmental stressors (including chronic stress from social inequities and racism) (Barber, 2020).

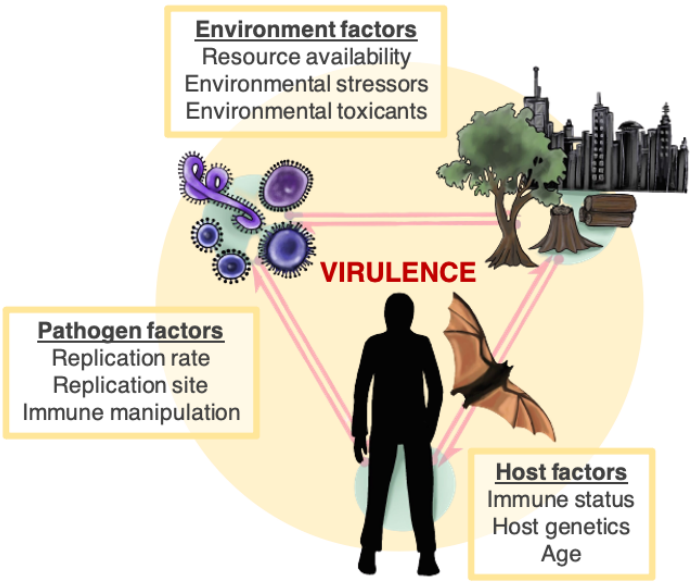

Figure 2: Disease Triangle of Virulence

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Box 2. Deriving R_0 maximization

We can look at a simplistic SI model to understand the math behind R_0 maximization. First, we set up our system of equations for the host-parasite system before mutation.

$$\frac{\mathrm{d}S}{\mathrm{d}t} = b - \beta SI - dS$$

$$\frac{\mathrm{d}I}{\mathrm{d}t} = \beta SI - dI - \alpha I$$

In this system, we have natural birth (b) and death (d), density dependent transmission (β), and virulence (α), but no recovery.

We can then solve for the ecological equilibrium of this system.

$$(1)\ \ S = \frac{b}{d}\ ,\ \ I = 0$$

$$(2)\ \ S = \frac{d+\alpha}{\beta}\ ,\ \ I = \frac{b}{d+\alpha} - \frac{d}{\beta}$$

The first equilibrium is simply when there is no infection in the system, so we focus on the second. This second equation is the ecological equilibrium of the system infected only by the resident strategy.

Now, we want to conduct an invasion analysis asking what mutant values (m) can invade the ecological equilibrium set by the resident strategy (r).

$$\frac{\mathrm{d}S}{\mathrm{d}t} = b - \beta_r SI_r - \beta_m SI_m - dS$$

$$\frac{\mathrm{d}I_r}{\mathrm{d}t} = \beta_r SI_r - dI_r - \alpha_r I_r$$

$$\frac{\mathrm{d}I_m}{\mathrm{d}t} = \beta_m SI_m - dI_m - \alpha_m I_m$$

To see when the mutant can invade, we determine the stability of the mutant-free equilibrium,

$$S = \frac{d+\alpha_r}{\beta_r}\ ,\ \ I_r = \frac{b}{d+\alpha_r} - \frac{d}{\beta_r}\ ,\ \ I_m = 0$$

.

The equilibrium is not stable when an emerging rare mutant can increase in number. This gives the invasion criteria.

$$\frac{\beta_m}{d+\alpha_m} > \frac{\beta_r}{d+\alpha_r}$$

In this simplistic example, the invasion criteria may be familiar as a form of R_0, the basic reproductive number. This means that the mutant with the highest R_0 can invade any population at equilibrium.

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Table 1. Empirical tests of virulence evolution theory			
Paper	**System**	**Virulence**	**Results**
The virulence and transmission trade-off			
(Anderson & May, 1982)	*Oryctolagus cuniculus* / Myxoma virus	Mortality rate	R_0 was maximized at an intermediate virulence that had slower recovery and mortality rates
(Mackinnon & Read, 1999)	*Mus musculus* / *Plasmodium chabaudi*	Body mass loss and anemia	Virulence and transmission stage density are both positively correlated with replication rate
(Mackinnon & Read, 2004)	*Homo sapiens* / *Plasmodium falciparum*	Mortality rate	Parasite fitness peaks at intermediate virulence values with higher parasite replication and lower mortality
(Jensen et al., 2006)	*Daphnia magna* / *Pasteuria ramosa*	Time to host death in an obligately killing, castrating parasite	Transmission stage production peaked at intermediate virulence
(Fraser et al., 2007)	*Homo sapiens* / HIV-1	Duration of asymptomatic infection	R_0 peaks at intermediate viral set point load and virulence
(Roode et al., 2008)	*Danaus plexippus* / *Ophryocystis elektroscirrha*	Emergence and mating probability, adult lifespan and fecundity	Parasite lifetime fitness peaks at intermediate replication rates
(Atkins et al., 2013)	*Gallus gallus domesticus* / Marek's disease virus	Host lifespan	R_0 peaks at intermediate virulence
(Doumayrou et al., 2013)	*Brassica rapa* / Cauliflower mosaic virus	Symptom severity	Virulence and transmission show a positive, saturating relationship, but the relationship with replication rate is not clear
(Tardy et al., 2019)	*Haemorhous mexicanu* / *Mycoplasma gallisepticum*	Body mass loss, symptom severity, and putative mortality rate	Virulence increases with parasite replication rate in isolates before, but not after host resistance evolution

(Bonneaud et al., 2020)	*Haemorhous mexicanu / Mycoplasma gallisepticum*	Host mortality and symptom severity	R_0 peaks at intermediate virulence, even when the relationship between transmission and virulence is not dependent on replication rate
(Acevedo et al., 2019)	Meta-analysis of multiple systems		Strong evidence of increasing relationships between virulence and replication and transmission and replication
Virulence evolution during epidemics			
(Berngruber et al., 2013)	*Escherichia coli* / bacteriophage lambda	Horizontal transmission through lysis (rather than vertical)	Virulent, lytic phage is strongly favored during competition at the start of an epidemic, but latent virus outcompetes it as the epidemic progresses
Virulence evolution in spatially structured populations			
(Kerr et al., 2006)	*Escherichia coli* / T4 coliphage	Competitive ability and productivity	Prudent strategies dominate with spatially restricted migration, while virulent phages dominate with global migration
(Boots & Mealor, 2007)	*Plodia interpunctella* / granulosis virus	Proportion of hosts infected in an obligate killer	Spatially structure selects for less infective, more prudent virus
(Berngruber et al., 2015)	*Escherichia coli* / bacteriophage lambda	Horizontal transmission through lysis (rather than vertical)	Latent, more prudent virus outcompetes lytic virus in spatially structured populations
Virulence evolution with environmental transmission			
(Ogbunugafor et al., 2013)	HeLa cells / vesicular stomatitis virus	Host cell death	There is a trade-off between transmission and the formation of environmentally persistent particles
(Wasik et al., 2015)	BHK cells / vesicular stomatitis virus	Plaque size	There is a trade-off between viral fecundity and the formation of environmentally persistent particles
(Walther & Ewald, 2004)	*Homo sapiens* / respiratory tract pathogens	Case fatality rate	Respiratory pathogens that survive longer in the environment are more virulent

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Virulence and transmission trade-offs acting at spillover

As we have outlined, theory on the virulence and transmission trade-off is based upon the idea that pathogens will be selected towards an optimal level of virulence within the host populations to which they are adapted (Cressler et al., 2016). Recently emerged zoonotic diseases do not have this evolutionary history with human populations and are therefore unlikely to be at their evolutionary optimum when they first emerge (Guth et al., 2019; Mollentze et al., 2020; Woolhouse et al., 2005). However, emerging pathogens may still be regulated by an underlying virulence and transmission trade-off. In meta-analyses of recently emerged viral zoonoses, excessively high virulence is associated with a lower R_0 (Brierley et al., 2016; Geoghegan et al., 2016; Guth et al., 2019) and this negative association supports the theoretical prediction that high virulence impedes pathogen fitness. Theory also predicts a cost to excessively low virulence, an effect that is not supported in these analyses (Anderson & May, 1982; Guth et al., 2019). However, this could easily result from discovery bias because we are unlikely to notice low-transmission zoonoses that cause only a few infections and have low virulence (Bonneaud & Longdon, 2020). As such, there is little evidence to not expect emerging diseases to be governed by trade-offs once they emerge into human populations.

What predicts the virulence of disease when it first gets to humans?

Emerging zoonoses vary widely in their virulence and transmission rates, but there are some pathogen and reservoir host characteristics that are associated with the pathogen's phenotype in humans (Geoghegan et al., 2016; Guth et al., 2019; Olival et al., 2017). In particular, meta-analyses of recently emerged viral zoonoses have supported phylogenetic trends in zoonotic potential (Guth et al., 2019). The phylogenetic distance between a pathogen's reservoir host and humans predicts the pathogen's probability of being zoonotic (Olival et al., 2017), virulence (Guth et al., 2019; Longdon et al., 2015), and R_0 in human populations (Geoghegan et

al., 2016; Guth et al., 2019). Mammalian hosts closely related to humans (e.g. primates) harbor zoonoses associated with lower human mortality and higher capacity for transmission, while more distantly related hosts (most notably, bats) harbor highly virulent zoonoses that appear to be relatively maladapted for human-to-human transmission (Guth et al., 2019).

These phylogenetic trends can be understood if pathogens from distantly related reservoir hosts have evolved replication strategies adapted to their reservoir host's more dissimilar immunology, physiology, and ecology (Guth et al., 2019; Mollentze et al., 2020). There may also be host orders with unique features in their biology beyond host dissimilarity that influence pathogen traits in humans (Brook & Dobson, 2015). Specifically, bats seem to harbor unusually virulent viruses (Brook & Dobson, 2015; Guth et al., 2019) which may, in part, result from their high viral tolerance selecting for high replication rates (Brook et al., 2020).

Transmission mode changes may cause shifts in virulence

Zoonotic pathogens often have different virulence in human hosts than in their reservoir hosts (Guth et al., 2019). However, pathogens that alter their transmission modes upon emergence may be expected to have especially large shifts in virulence (Ewald, 1991). Most pathogens have multiple possible modes of transmission, where their primary mode is determined by factors like host social behavior and the environment (Antonovics et al., 2017). Pathogens can undergo immediate shifts in transmission route upon emergence when human behavior promotes the primary use of transmission routes not preferred in their reservoir hosts or when the receptors that they bind to are located in different tissues (Antonovics et al., 2017). This can lead to immediate shifts in virulence due to changes in pathogen inoculum size and anatomical site of infection (Leggett et al., 2012; McMahon et al., 2018). Over longer evolutionary time scales, different transmission pathways may create novel selection pressures on virulence (Ewald, 1991).

252 Thus, changes in a parasite's dominant mode of transmission during emergence can lead to both
253 immediate changes in and selection pressures for future changes in virulence.
254
255 **Virulence and transmission relationships are likely maladapted in emerging pathogens**
256 Pathogen virulence and capacity for transmission in humans loosely trade-off in a meta-
257 analysis of zoonotic viruses (Guth et al., 2019). Despite this trend, there is a substantial amount
258 of noise in the relationships between virulence and transmission. Some of this noise is likely due
259 to the fundamental complication of predictive evolution that each pathogen will have a unique
260 trade-off curve dependent on the nuances of its biology (Ebert & Bull, 2003). However, entirely
261 maladapted phenotypes also exist below the trade-off curve (Bull & Ebert, 2008; Shoval et al.,
262 2012). Simply, novel zoonotic pathogens can be bad at both transmission and virulence. Overall,
263 then, pathogens will vary in virulence and transmission because they have unique trade-off
264 shapes that predict different optimum values and because they can be maladapted below the
265 trade-off.
266 The concept of Pareto fronts describes such scenarios where phenotypes can be in the
267 region of sub-optimal phenotype space below the trade-off front (Shoval et al., 2012). The trade-
268 off front (or Pareto front) separates these accessible, maladapted phenotype combinations from
269 impossible, ideal phenotypes (Li et al., 2019; Shoval et al., 2012). In the sub-optimal region below
270 the Pareto front, improvements in one trait may not affect the other trait as simple adaptations
271 can be made before costs are incurred. Applied to virulence evolution, this concept means that
272 recently emerged diseases, even if broadly regulated by trade-offs, may select for no-cost
273 improvements in transmission that do not affect or can actually decrease their virulence (See
274 Figure 3a) (Bull & Ebert, 2008). The relationship between virulence and R_0 in recently emerged
275 zoonotic viruses seems to display such a Pareto front where phenotypes exist below, but not
276 above, a trade-off front (See Figure 3b).

For an illustrative example of how virulence and transmission can break trade-offs in maladapted zoonosis, we can use H5N1 as a case study (Wasik et al., 2019). Concern over the pandemic potential of highly pathogenic avian H5N1 influenza A led to two experimental evolution studies examining the virus's ability to evolve respiratory droplet transmission in a ferret model system (Herfst et al., 2012; Imai et al., 2012). Both studies found that the virus could evolve respiratory droplet transmission, which would increase its transmission rate. However, this higher transmission rate actually correlated with substantial decreases in virulence (Herfst et al., 2012; Imai et al., 2012). This was because avian influenzas recognize a sialic acid (Siaα2,3Gal) that is found in ferrets' (and humans') lower respiratory tracts while human influenzas recognize a sialic acid (Siaα2,6Gal) found in the upper respiratory tract (Herfst et al., 2012). In the lab, H5N1 was able to evolve Siaα2,6Gal recognition and localize to the upper respiratory tract tissues that allowed for droplet transmission. This change in replication site led to more efficient transmission, but also lower host mortality despite no selection against virulence in the experiment (Herfst et al., 2012; Imai et al., 2012). Notably for our understanding of the virulence and transmission trade-off, these changes were dependent on changes in replication site, not replication rate. In some sense, these no-cost transmission improvements could only happen because the virus was so maladapted to mammalian hosts that it was using suboptimal binding sites. After these no-cost adaptations brought the virus to the Pareto front, further adaptation would have to involve changes to transmission and virulence that trade-off with each other through processes like replication rates.

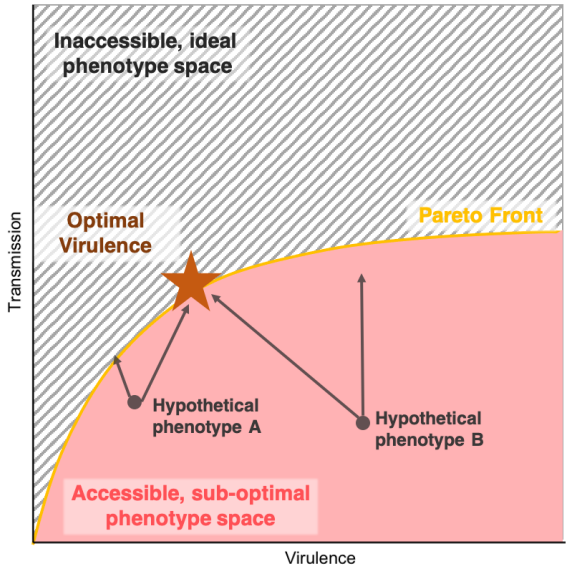

Figure 3a. Conceptual Diagram of the Pareto front between virulence and transmission. Possible phenotypes can be selected to improve transmission along any pathway within the accessible phenotype space. Since each pathogen's function determining their virulence and transmission trade-off varies, we cannot know where a hypothetical phenotype sits below the Pareto front. Selection for improved transmission can therefore involve decreases, no changes, or increases in virulence depending on the pathogen's starting point and mutational availability.

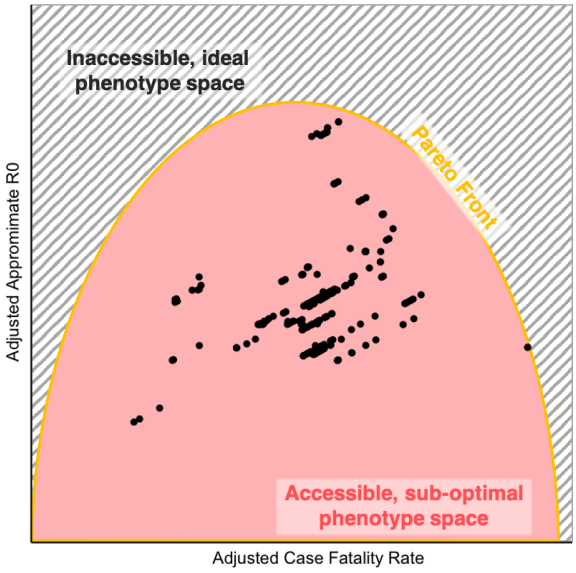

Figure 3b. Recently emerged viral zoonoses follow a Pareto front of virulence and reproductive capacity. Data is from a published dataset of recently emerged viral zoonoses from mammalian hosts (Guth et al. 2019). Approximate R0 is classified from 1 (no recorded human to human transmission) to 4 (endemic transmission) and adjusted by virus family and number of citations. Case fatality rate is adjusted by virus family and number of citations. Dots represent adjusted CFR and R0 values for individual epidemics of different viral zoonoses.

Do we expect to see adaptive evolution of transmission and virulence in recently emerged diseases?

While there are certainly selection pressures on recently emerged zoonotic pathogens, this does not necessarily mean that there will be adaptive evolution (Grubaugh, Petrone, et al., 2020; MacLean et al., 2020). A key tenant of evolutionary theory is that selection pressures must act through a background of stochasticity and drift to result in adaptive evolution (Crow & Kimura, 2009). As small population sizes mean that both stochasticity and drift are relatively strong, the inevitably small population of infected individuals at the start of an epidemic means that these factors are likely to overwhelm selection and determine the spread of mutants (Hartl & Clark, 1997).

Stochastic effects are additionally exasperated by the existence of founder effects during epidemic range expansions resulting in spatial stochasticity analogous to genetic drift (Slatkin & Excoffier, 2012). Thus, founder effects and variation in transmission due to host behavior and stochasticity likely determine the fate of mutants at the start of epidemics (MacLean et al., 2020). However, as the population size of infected individuals increases or if there are mutations of large enough effect size, the balance between selection and stochasticity may shift towards selection and result in adaptive evolution.

Finally, the adaptive evolution of acute, respiratory pathogens may additionally be constrained by the small bottleneck sizes of transmission events, which also increase stochasticity (McCrone et al., 2018; McCrone & Lauring, 2018). The normally short infectious periods of acute diseases mean that only limited amounts of mutation and selection can occur before transmission. Small bottleneck sizes mean that only a few genetic variants are transmitted. Together, these factors mean that it is less likely for an acute, respiratory virus to have enough time within a host to generate adaptive mutations and select on those variants strongly enough for them to reach high enough frequencies to transmit through tight bottlenecks to other individuals (McCrone et al., 2018). This can impede adaptive evolution at the population level (Morris et al., 2020). This may mean that individuals with chronic infections are especially important for adaptive evolution in acute, respiratory pathogens as they have longer infectious periods that allow for the fixation of beneficial mutations (Rambaut et al., 2020; Xue et al., 2017).

What are the selective pressures on transmission and virulence in recently emerged diseases?

Standard eco-evolutionary theory assumes that ecological and evolutionary time scales are decoupled such that ecological equilibrium is reached before new mutants invade (Metz et al., 1995). Epidemics are definitionally not at ecological equilibrium and high mutation rates mean

that new variants are likely to arise early in epidemics (Bull & Ebert, 2008). Therefore, the assumptions of decoupled timescales must be relaxed to examine how selection pressures on virulence and transmission change over the course of an epidemic (Lenski & May, 1994).

Selection on virulence and transmission during epidemics

Selection pressures on virulence and transmission during epidemics can be explored by using models that do not assume separation of time scales, often using population genetic approaches (Bolker et al., 2010; Bull & Ebert, 2008; Day & Gandon, 2007; Day & Proulx, 2004; Lenski & May, 1994). These models allow for the existence of multiple simultaneous mutants so that the competitive fitness of each can be assessed over shifting ecological conditions in time. They show that strategies with higher transmission rates (betas) and virulence can be selected during epidemic growth stages, despite R_0 optimized (intermediate virulence) strategies dominating at endemic equilibrium (Day & Proulx, 2004; Lenski & May, 1994). This is because strategies with higher transmission rates spread fastest at the start of the epidemic when the density of susceptible hosts is high (Day & Proulx, 2004; Lenski & May, 1994).

Intuitively, these results can be explained as: an infected host during the early stages of an epidemic encounters mostly susceptible hosts, so strains with higher transmission rates will have faster population growth rates since they have shorter generation times than strains with higher R_0s (but lower transmission rates) that produce more secondary infections more slowly over a longer infectious period. Therefore, improvements in transmission rate are the most important at the start of an epidemic and can be selected for even if they increase virulence. This also demonstrates that the high density of susceptible hosts early in epidemics crucially influences selection pressures (Bull & Ebert, 2008; Cressler et al., 2016; Day & Proulx, 2004; Lenski & May, 1994).

Selection on virulence and transmission in structured populations

Simple virulence evolution trade-off theory assumes that transmission happens randomly in a homogeneously mixing population (Cressler et al., 2016). However, natural populations almost always have heterogeneous mixing patterns due to spatial structure and social networks (Boots & Sasaki, 1999; van Baalen, 2002). In these structured populations, transmission mostly happens between neighboring individuals. This can lead to 'self-shading' where highly infectious strains rapidly deplete their local susceptible populations and compete for available hosts with related strains (Boots & Sasaki, 1999; Boots & Sasaki, 2000; Lion & Boots, 2010). Both these components of 'self-shading', the ecological clustering of infected individuals and the genetic clustering of related strains, slow the rate of spread of highly infectious strains. On the other hand, less infectious strains maintain higher local densities of susceptible individuals and have higher onward transmission (Boots & Sasaki, 1999; Boots & Sasaki, 2000; Lion & Boots, 2010). Thus, structured host populations select for lower pathogen infectivity and virulence at endemic equilibrium.

However, the high availability of susceptible hosts at the start of an epidemic is likely to reduce the impact of self-shading. Instead, we see that pathogens need to have higher transmission rates to seed an epidemic in a spatially structured population than in a well-mixed one (Keeling, 1999). Before ecological equilibrium, the invasion front of a spatially structured epidemic also has a high local supply of susceptible hosts. This leads to a dynamic where virulent, high beta strains are selected at the invasion front and then are succeeded by more prudent strategies as the local dynamics approach equilibrium (Griette et al., 2015; Lion & Gandon, 2016). Overall then, it is possible that structure in host populations temporarily selects for higher virulence while the epidemic is spreading through mostly susceptible populations. However, if there are also trade-offs where high virulence impedes host movement, then the spatial front of

384 the epidemic might instead have lower virulence (Hawley et al., 2013; Osnas et al., 2015). As
385 such, it is unclear how population structure and movement overall will select emerging pathogens.
386
387 **Selection on virulence with environmental transmission**
388 Simple virulence evolution trade-off theory assumes that pathogens only transmit by direct
389 contact between hosts. However, many pathogens also transmit through the environment
390 (Bonhoeffer et al., 1996; Ewald, 1983; Gandon, 1998; Kamo & Boots, 2004). The 'curse of the
391 pharaoh' hypothesis suggested that parasites can have higher virulence when they transmit
392 through the environment because transmission is not linked to the host's infectious period (Ewald,
393 1983). However, at ecological equilibrium, environmental transmission can select for higher
394 virulence only if hosts can be multiply infected or if transmission can happen from environmental
395 pools after host death (Day, 2002; Day & Gandon, 2006; Gandon, 1998). Propagule survival in
396 spatially structured populations may actually increase self-shading and select for even lower
397 virulence (Kamo & Boots, 2004).
398 Importantly, environmental transmission also selects for higher virulence during the
399 epidemic stage if propagule dynamics are faster than host dynamics (Bonhoeffer et al., 1996).
400 This result holds even if hosts are singly infected and do not transmit after death because it
401 instead relates to the relative speed of pathogen population growth rates (Bonhoeffer et al., 1996;
402 Lenski & May, 1994). Under 'curse of the pharaoh', more virulent strategies with shorter infectious
403 periods will be more represented in the environmental reservoir and will therefore have higher
404 population growth rates when the susceptible density is high. Overall then, it is likely that
405 environmental transmission will select for higher virulence in epidemics (Bonhoeffer et al., 1996;
406 Day, 2002; Day & Gandon, 2006; Ewald, 1983; Gandon, 1998). However, it can be costly to make
407 environmentally persistent particles if they require more host resources or impede attachment to
408 host cells (Ogbunugafor et al., 2013). This can alter the dynamics of 'curse of the pharaoh' models

409 and potentially lead to bistability or branching resulting in sudden shifts and diversity in virulence
410 (Boldin & Kisdi, 2012; Caraco & Wang, 2008; Roche et al., 2011).
411
412 **Selection on virulence with antigenic escape**
413 Finally, simple virulence evolution trade-off theory assumes that recovered hosts are fully
414 immune such that host immunity does not wane and pathogens do not evolve to escape such
415 immunity. However, some, but not all, viral pathogens exhibit antigenic evolution to escape
416 neutralizing antibodies conferred by previous infections or vaccines (Drexler et al., 2014; Kennedy
417 & Read, 2017; Mclean, 1998; Rambaut et al., 2008; Wong et al., 2017). Notably though, selection
418 for antigenic or vaccine escape evolution is significantly slower and less efficient than for drug
419 resistance—likely due to differences in the timing and breadth of selective pressures (Debbink et
420 al., 2017; Kennedy & Read, 2017; Morris et al., 2020). When antigenic escape occurs, however,
421 it means that recovered individuals are newly susceptible to evolved strains and essentially
422 'resets' the timescale of an epidemic by replenishing the density of susceptible hosts. This effect
423 had been postulated to transiently select for transmission-maximizing strategies with higher
424 virulence (Bull & Ebert, 2008), but has recently been shown to select for the long term persistence
425 of more acute, highly transmissible and virulent pathogens (Sasaki et al., 2021). However, the
426 mutations conferring antigenic escape likely trade-off with other pathogen traits like receptor
427 binding avidity, folding, and expression and therefore may constrain the possible virulence and
428 transmission phenotypes for such mutants (Greaney et al., 2020; Hensley et al., 2009).
429
430 **Other factors shaping selection on virulence**
431 Many other factors influence the evolution of virulence and have been reviewed elsewhere
432 (Cressler et al., 2016). In brief, multiple infection or co-infection may select for more virulent
433 pathogens due to within-host competition for resources (Alizon & van Baalen, 2008). Host

434 demographic features like immigration and density dependent mortality or fecundity may also alter
435 selection on virulence (Cressler et al., 2016). Additionally, host heterogeneities like age (Iritani et
436 al., 2019), genetic diversity (Osnas & Dobson, 2012; Regoes et al., 2000), and resistance
437 (Gandon, 2004) may select for virulence optimized on certain types of host.
438
439 **How might public health measures shape selection on virulence?**
440 The question of whether public health measures can purposely or inadvertently drive
441 pathogen evolution naturally arises when discussing virulence evolution. It is likely to be very
442 difficult to purposefully manage virulence evolution because it is so difficult to fully predict (Ebert
443 & Bull, 2003). For one, zoonotic pathogens can evolve in unpredictable ways if they start below
444 the Pareto front of the virulence and transmission trade-off. Additionally, selection pressures on
445 virulence are dependent on trade-offs that vary for each disease and moreover host population
446 characteristics that change rapidly. Finally, as we have discussed, selection pressures on
447 virulence are likely to be weak compared to stochastic effects at the start of epidemics.
448 Public health measures intentionally driving the evolution of virulence may therefore be
449 quixotic fantasies for emerging diseases. However, we can gain insight into how public health
450 measures can inadvertently select on virulence. Non-pharmaceutical public health interventions
451 for epidemics primarily aim to decrease transmission and therefore either stop the epidemic or
452 slow it until vaccines and treatments can be developed (Lai et al., 2020). This decreases the total
453 number of infected individuals, which will have the greatest impact on the total mortality burden
454 of any epidemic (Day et al., 2020).
455 However, some of these interventions may also contribute to the selection pressures
456 acting on the pathogen (Day et al., 2020; Ebert & Bull, 2003). First, increased environmental
457 sanitation raises the propagule death rate in the environment, thus potentially selecting for lower
458 pathogen virulence under the 'curse of the pharaoh' hypothesis (Bonhoeffer et al., 1996). Second,

decreased travel and extra-household contacts should alter the spatial and social structure of the population to make a more structured transmission network (Boots & Sasaki, 1999). Third, quarantine of symptomatic individuals may select for decreased or altered symptoms (Knell, 2004; Saad-Roy et al., 2020). Finally, vaccines can sometimes create selection pressures on pathogens with potential evolutionary impacts to consider (Kennedy & Read, 2020). However, recent models have explored potential vaccine induced selection for proposed Sars-CoV-2 vaccines and suggest that they are unlikely to select for higher virulence (Miller & Metcalf, 2020).

While the most human mortality will be prevented by simply preventing transmission, considering the effects of control measures on virulence evolution can, in principle, lead to better epidemic management (Day et al., 2020). Understanding host population characteristics creating strong selection pressures for high transmission strategies could help distribute public health effort if there are limited resources (Day et al., 2020). Importantly, weak epidemic control measures that allow for extended transmission in humans increase the evolutionary potential of zoonotic pathogens because they allow for stronger selection pressures and more mutations (Day et al., 2020). Thus, the best evolutionary management practice for an epidemic of a zoonotic infectious disease would be to suppress transmission using strong, rapid public health interventions.

Box 3. Future Research Questions

There are several gaps in our understanding of the patterns and predictors of virulence evolution that require interdisciplinary, integrative approaches across often siloed subfields.

- What are the costs to transmission in human populations? Human pathogens are fairly rarely limited by host mortality, so models that purely define virulence as 'the additional mortality rate due to infection' are often inappropriate for virulence evolution in human

populations. This gap is currently hindering the uptake of trade-off theory amongst applied virology and medical fields (Bull & Lauring, 2014) and several other trade-offs like time to recovery and sickness behavior may be more applicable for most human pathogens (Alizon, 2008; Anderson & May, 1982; Ewald, 1994). Effort should be put towards determining the empirical evidence for such trade-offs and exploring their consequences for evolutionary theory.

- What is the distribution of mutational fitness effects for pathogen traits like transmission and virulence in emerging zoonotic diseases and what are their trade-offs (or lack thereof)? Many studies of mutational fitness effects use simple metrics of fitness that do not capture how different components of fitness may trade-off (Sanjuán, 2010; Visher et al., 2016), but see (Greaney et al., 2020). Unmeasured evolutionary constraints and trade-offs may alter the distribution of mutational fitness effects outside of simple laboratory conditions (Visher & Boots, 2020). As selection may act on different fitness components differently at various time scales, being able to disentangle their relative contributions is important to build stronger predictive theory.
- How do selection pressures and their strengths relative to stochasticity vary across within host, between host, and population level scales? Many models do not include the heterogeneous selection pressures that arise from changes in pathogen population sizes at transmission, infection progression across tissues, temporally varying immune pressures, and heterogeneous hosts (Visher & Boots, 2020). Models that include these nuanced empirical circumstances can sometimes better describe population level evolutionary dynamics (Mideo et al., 2008; Morris et al., 2020; Ogbunugafor et al., 2010).

477

Conclusion

Novel zoonotic pathogens emerge into the human population maladapted to human hosts and, although it is difficult to predict their emergence, pathogen and reservoir host traits can loosely predict their virulence and transmission phenotypes (Guth et al., 2019; Olival et al., 2017). Broadly, virulence is thought to trade-off with transmission leading to an optimal, intermediate level of both. However, maladapted virulence and transmission phenotypes may start below the Pareto front, so selection for transmission can have decoupled effects on virulence (Bull & Ebert, 2008). Selection pressures on pathogens are weak compared to drift during the highly stochastic early epidemic period, but can become relevant if epidemic control fails and extended transmission occurs (Hartl & Clark, 1997). A nuanced body of theory describes the selection pressures acting on pathogen transmission and virulence (Cressler et al., 2016). These selection pressures follow the three Ts—trade-offs, transmission, and time scales. Trade-offs between traits mean that pathogens are selected to balance the benefits of transmission with the costs of virulence, but the relative balance depends on the time scale of the epidemic (Anderson & May, 1982; Bull & Ebert, 2008). When the density of susceptible hosts is high early in the epidemic, pathogens are selected for higher transmission rates even if they trade-off with higher virulence (Bull & Ebert, 2008; Lenski & May, 1994). To predict how a pathogen's virulence will evolve then, we must understand the fitness impacts and trade-offs of transmission-improving mutations and the population structure of the host (Day et al., 2020). This makes virulence evolution an academically interesting topic with a rich body of theory surrounding it, but no universal predictions (Ebert & Bull, 2003). However, this will not be a problem if strong, rapid public health measures suppress transmission early on since this will both decrease the evolutionary potential of such pathogens and decrease the total mortality burden by limiting the number of people infected.

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Author Contributions

All authors researched and edited the paper. EV and MB conceptualized and wrote the paper.

Data accessibility

No novel data is used in this manuscript; data used is publicly available from (Guth et al., 2019). The annotated R script used for analysis is in the supplementary materials.

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Competing Interests

We declare no competing interests.

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