# Title: Transgenerational virulence: Maternal pathogen exposure reduces offspring fitness

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**Abstract:** There is intense interest in understanding the degree of damage caused by a pathogen. However, despite abundant evidence that effects of stressors can carry across generations, studies of pathogen virulence have focused almost exclusively on a single generation. Here, we describe transgenerational virulence, where parental exposure to a pathogen reduces offspring fitness. *Daphnia* host exposure to a microsporidian pathogen significantly reduced lifespan, and in some cases fecundity, of unexposed offspring. These effects carried to the granddaughters, which were smaller if the grandmother had pathogen exposure. A parameterized mathematical model translated these impacts to the population level, finding that transgenerational virulence often reduces host density. Given widespread prior evidence for transgenerational effects of predators, resources, and other factors, we propose that transgenerational virulence may be common.

**One-Sentence Summary:** Virulent effects of pathogens can carry across generations, exacerbating population-level impacts of pathogen outbreaks.

**Main Text:** By definition, pathogens harm their hosts, and the degree of damage done to the host by the pathogen (known as "virulence") is a major focus of research on host-pathogen interactions. Virulence is traditionally investigated within a generation, measuring the cost of exposure or infection to the exposed/infected individual. However, for many other stressors there is strong evidence of transgenerational effects; for example, maternal predation exposure impacts offspring behavior and growth (*1–4*) and food restriction can impact offspring size and survival (*5–8*). Still, despite the clear evidence of transgenerational effects of other stressors and the intense interest in understanding pathogen virulence, the potential for transgenerational virulence — that is, reduced fitness of the offspring of exposed or infected hosts — has been overlooked.

While transgenerational virulence has been overlooked, transgenerational effects of infection are well established. In particular, hosts that are exposed to a pathogen can reduce the susceptibility of their offspring to that pathogen or different pathogens, a phenomenon known as transgenerational immune priming (*9–12*). Moreover, offspring of exposed hosts can have altered immune profiles (*13–15*). Thus, it is clear that impacts of exposure to pathogens can carry across generations. A few studies have explored the potential for negative effects of infection on offspring survival (*16*, *17*) or population growth (*11*). However, there has yet to be a clear example of transgenerational virulence due to pathogen infection or exposure. Demonstrating transgenerational virulence would require showing that: first, the impact of infection occurs in offspring that are not themselves infected (which differs from an example like Zika virus, where the offspring becomes infected (*18*)); second, offspring fitness (e.g. lifespan or reproduction) is reduced; and, third, the impact is not because the offspring were unable to complete development as a result of the maternal infection (as occurs with SARS-CoV-2 infections where the placenta is damaged (*19*)).

Here, we demonstrate that exposure of a host to a pathogen reduces fitness of her unexposed and uninfected offspring. We do this using laboratory experiments with *Daphnia* hosts, which are a model system for understanding host-pathogen interactions (*20*). One advantage of the *Daphnia* system is that individual genotypes can be maintained as asexual clonal lines, with mothers producing daughters clonally. We used three genotypes of *D. dentifera* and a microsporidian, *O. pajunii*, that infects epithelial cells of the host gut. Individuals of each of the three genotypes were exposed either to *O. pajunii* or to a control solution (see Supplementary materials and Fig S1). After exposure, we collected offspring and reared those individuals for 20 days, tracking offspring mortality and reproduction; these offspring were never exposed to a pathogen and we describe them as uncompromised offspring (animals with unexposed mothers) or compromised offspring (animals with exposed mothers). In addition, to test for transgenerational immune priming, we also collected a second set of offspring of exposed and control mothers; we exposed these offspring to *O. pajunii*, then quantified whether they became infected and the level of infection.

Exposure of mothers to the pathogen significantly reduced the lifespan of their uninfected offspring (Figure 1A; hazard ratio of compromised to uncompromised individuals: 3.285, 95% CI 1.165 to 9.265, p=0.025). Moreover, while there was not an effect of maternal exposure on overall offspring reproduction, offspring of the genotype whose lifespan was most significantly reduced by maternal exposure (the "S" genotype) suffered reduced fecundity (Figure 1B; $ChiSq_{(1)}$=8.47, p=0.004) in addition to a shortened lifespan. Offspring of compromised individuals that survived to reproduce produced smaller neonates (Figure 1C, $F_{(1,43)}$=4.147, p=0.048). In contrast, we did not see evidence of transgenerational immune priming: maternal

exposure did not impact infection prevalence or intensity in offspring that were exposed to the pathogen (prevalence: $Rao_{(1,58)}$=1.111, p=0.292; intensity: $F_{(1,21)}$=2.43, p=0.134).

Studies on transgenerational effects of other stressors point to potential mechanisms that might underlie these empirical findings. First, molecular mechanisms often influence transgenerational shifts in response to stressors; for example, in Polpay sheep, paternal diet stress led to epigenetic changes that impaired growth and reproductive phenotype in offspring (*21*) and toxicant exposure in *C. elegans* induces transgenerational decreases in fitness due to epigenetic regulation (*22*). Second, organisms can shift energetics in response to stressors; for example, altering reproduction under conditions of environmental stress (or parasitism) is a well characterized strategy to maximize fitness. While this is commonly seen as changes in the number of offspring (*23*, *24*), predator signals in the maternal environment during egg development can cause reduced fat content among *Daphnia* eggs, signaling reduced maternal investment (*25*, *26*). Additionally, these two mechanisms may combine: in fruit flies, high sugar maternal diets altered body composition of offspring and modified metabolic gene expression, with fitness impacts carrying throughout the offspring lifespan (*27*). Reducing reproductive investment after pathogen exposure may provide more resources for immune response, a key strategy for combating pathogens like *O. pajunii*, which is of relatively low (within generation) virulence and does not pose an immediate mortality risk. We hypothesize that exposure to the pathogen caused mothers to reduce investment in embryos, yielding offspring with reduced early survival. This would result in the appearance of similar reproductive success for the parent generation, with costs of exposure manifesting as transgenerational virulence in the offspring generation. In our system, the most notable impact was on early mortality; this is consistent with the paradigm that any reduction in fitness due to reduced maternal investment would manifest early in life (*28*).

To explore the implications of transgenerational virulence on host-pathogen dynamics at the host population level, we developed single host-single pathogen mathematical models with susceptible and infected uncompromised and compromised individuals and an environmentally transmitted pathogen. We use our model to explore how changes in the mortality, reproductive rates, or infection rates of compromised individuals impact total host density, infected host density, and infection prevalence.

Our model predicts that, if transgenerational virulence manifests as increased mortality or decreased reproduction of offspring of infected mothers, then it will reduce total host density, infected host density, and infection prevalence in most systems (Figure 2, Figure S6); in other words, transgenerational virulent effects on reproduction and survival harm both the host and the pathogen at the population level. If transgenerational virulence manifests as increased infection rates by offspring of infected mothers, then it will harm the host population but benefit the pathogen in most systems: increased infection rates for compromised individuals decreased total host density, while infected host density and infection prevalence both increased (Figures S2-S4, S6-S8). When parameterized for the *Daphnia*-MicG system, the model predicted that the observed increase in mortality of susceptible and infected compromised individuals due to transgenerational virulence reduced total host density by 20% (Fig. 2A), infected host density by 33% (Fig. 2B), and infection prevalence by 17% (Fig. 2C). Additional details about the model, the above predictions, and exceptions are provided in the Supplementary Material.

Our model highlights the potential impact of transgenerational virulence on a community. Host density is a significant factor in spillover risk (*29*), so the predicted decreases in total host

density may lead to decreases in spillover. Moreover, transgenerational virulence reduced infected host density, which should further reduce spillover risk in systems with density-dependent transmission (*30*). Thus, in cases where a host population is of conservation concern, transgenerational virulence might have mixed impacts at the population level: if the impacted host is the one of conservation concern, transgenerational virulence should exacerbate the impacts of infection, but if infections from the impacted host spillover to a host of conservation concern, transgenerational virulence should reduce spillover risk.

While there was a clear overall pattern of transgenerational virulence for all three genotypes in our study, the manifestation differed somewhat among genotypes. The impact on daughter lifespan and reproduction was particularly strong for one genotype ('S'), the effect on granddaughter size was particularly strong for another genotype ('D'), and the third genotype ('B') suffered intermediate impacts on lifespan, reproduction, and granddaughter size. This difference among genotypes in transgenerational impacts strongly resembles the variation shown by *Drosophila* genotypes in transgenerational responses to environmental stressors (*31*). These differences have the potential to scale up to host population-level differences in the ability to compensate for transgenerational virulence driven increases in mortality (*32*), with potential implications for host population genetic composition and infection prevalence – providing further evidence of the importance of considering host life history in disease ecology (*33*).

Our experiment focused on transgenerational impacts of pathogen exposure, rather than on infection. Here, offspring of exposed mothers suffered reduced fitness even though there were no obvious signs of infection in the mothers at the time the offspring were produced. This is consistent with earlier work that found reduced fitness in bumblebees within the same generation, where individuals who were exposed to a sham infection that stimulated the immune system had greatly reduced survival (*34*). Thus, our work adds to evidence that virulence costs may start accumulating upon exposure, and extends this to show those impacts can span generations.

Here, we demonstrated that exposure to a microsporidian pathogen has detrimental effects on the fitness of offspring of the exposed hosts. This transgenerational virulence manifested as decreased survival and reduced fecundity of offspring of exposed mothers, as well as smaller granddaughters. These individual-level transgenerational impacts of pathogen exposure have consequences at the population-level: our model reveals that transgenerational virulence can notably decrease host density, infected host density, and infection prevalence. Given the widespread prior evidence for transgenerational effects of stressors, we propose that transgenerational virulence is likely to occur in other host-pathogen systems, with consequences for both hosts and pathogens.

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**Supplementary Materials**
Materials and Methods
Supplementary Text
Figs. S1 to S9
Tables S1 to S4
References (*35-45*)

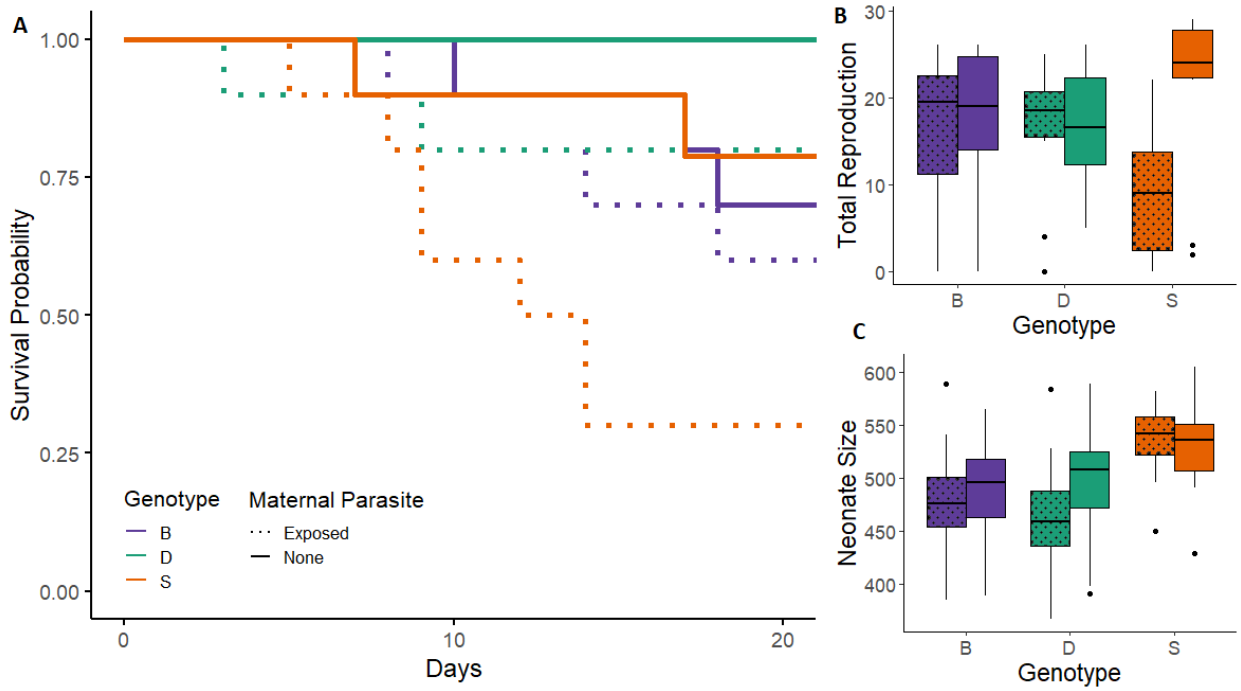


**Fig. 1. Daughters of exposed mothers had reduced lifespan and produced smaller neonates, and, for one genotype, had reduced reproduction.** (**A**) Survivorship curves for the three genotypes; each color represents one genotype, with solid lines representing offspring of mothers that had not been exposed to *O. pajunii* and dashed lines representing offspring whose mothers were exposed to the pathogen. (**B**) for the S genotype (orange), daughters of exposed mothers (dotted box) produced fewer offspring than daughters whose mothers were not exposed to the pathogen; for all three genotypes, the dotted boxes indicate daughters of exposed mothers. Panel (**C**) Neonates produced by daughters of exposed mothers (dotted box) were smaller than those produced by daughters of unexposed mothers; this indicates that transgenerational effects of infection carry through to granddaughters of exposed mothers. Note: none of the daughters in

this part of the experiment were exposed to the pathogen; thus, these effects are not due to within generation virulence.

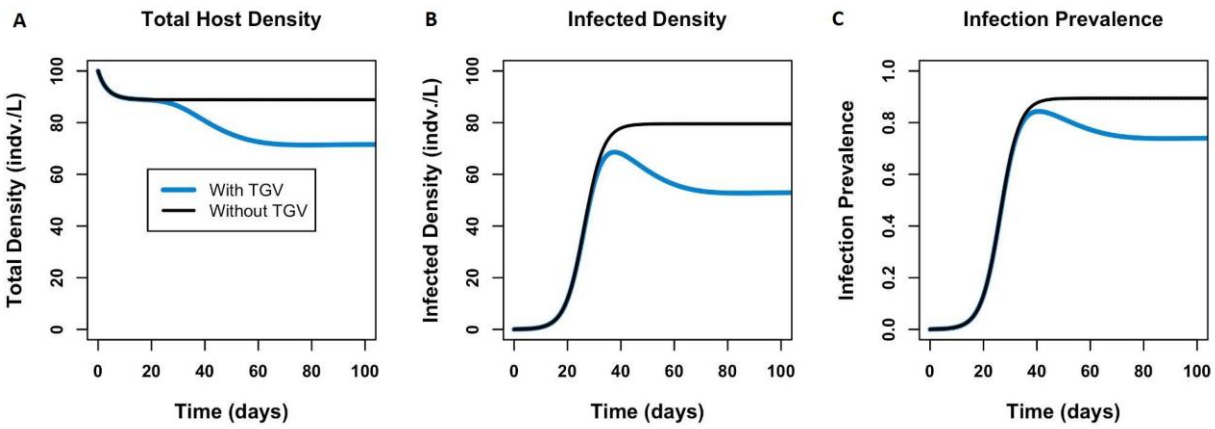


**Fig. 2. Population level effects of Transgenerational virulence**. Transgenerational virulence (TGV) notably reduces total host density (**A**), infected host density (**B**), and infection prevalence (**C**). Thinner black lines show the times series from a parameterized model where there is no TGV; thicker blue lines show a model with TGV, where offspring of infected individuals have higher mortality.