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THE INFLUENCE OF MULTIPLE INFECTIONS ON INFLUENZA VIRULENCE EVOLUTION

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Multiple infections occur when individual hosts are infected by more than one source of a pathogen. Theoretical models predict that multiple infections increase pathogen virulence and accelerate adaptation to the host, but very little experimental work has tested these predictions. Recent research shows that up to 66% of infections with influenza A virus (IAV) could be the result of multiple infection events. The apparent common nature of multiple IAV infections coupled with the possibility that they lead to increased viral virulence and fitness suggests a serious exploration of the subject is needed.

We have designed an experiment in which we serially passaged IAV in male and female BALB/c and C57BL/6 mice while varying the number of donor hosts for ten rounds of 3-day passages. IAV was experimentally transmitted between single hosts (1X) or the virus from two or four hosts was combined before every subsequent round (2X and 4X, respectively). Once all serial passages were completed, the various viral lines were tested by infecting groups of mice and measuring their weight loss for ten days. Mice that reached 75% of their initial weight were euthanized. Other groups of test mice were euthanized at day 3 post-infection in order to collect virus for viral titration. An additional test phase experiment was performed in which the single-host passage lines were combined at the end and used to infect groups of mice in order to simulate a single multiple infection event with independently evolving viral lineages (1XPooled).

We found that multiple infections increase the average virulence of IAV and increasing amount of donors leads to higher levels of virulence and mortality (LMM; 2X $p=0.025$; 4X $p<0.001$). Furthermore, we found that a single multiple infection event with independently evolving (1XPooled) viral lineages leads to a high virulence infection, and this treatment had the highest virulence levels measured (LMM; $p<0.001$). Interestingly, viral fitness did not increase significantly with the multiple infection treatments, which suggests that higher virulence is not necessarily a product of increased viral fitness. Further experiments are being planned that test the mechanisms that lead to these substantial increases in virulence.

This work represents the first experimental exploration of the effects of multiple infections in a vertebrate host-pathogen system. We have demonstrated that multiple infections accelerate virulence evolution and that a single multiple infection event can lead to a large increase in pathogen virulence. These observations might be applicable to many other pathogen types and have epidemiological consequences ranging from informing preventative isolation measures for infected individuals (for example in hospitals) to intensive agricultural settings, which are associated with inter-species pathogen transmission events that can lead to pandemics.