


INSIGHTS

Playing dirty with virus transmission

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In this issue of *JEM*, Fay et al. (2021. *J. Exp. Med.* <https://doi.org/10.1084/jem.20211220>) cohoused dirty pet store mice and rats with clean laboratory mice to gain insights into infection dynamics, discover new viruses, and identify relationships between viruses and the microbiome.

The global virome is enormous, with >1 million different viruses capable of infecting birds and mammals (Carroll et al., 2018). Emerging pathogens such as Zika virus and the ongoing COVID-19 pandemic have highlighted the importance of identifying those viruses among this vast number with zoonotic potential. There is considerable interest in identifying barriers for transmission to a different host species and understanding the subsequent process of adaptation to the new host. However, our current transmission models are often inadequate as they lack a natural reservoir, use single high infectious doses, and non-physiological routes of inoculation. In addition, many studies are limited to one pathogen and one host species, neglecting any potential interaction between different microbes. A few natural transmission models have been established in recent years, including wildling mice (laboratory mice born to wild mothers) and rewilding (release of laboratory mice into an outdoor enclosure; Lin et al., 2020; Rosshart et al., 2019; Yeung et al., 2020). These approaches represent innovative ways to investigate long-term exposure or colonization by members of the microbiota while still being able to take advantage of genetic mouse models. It is also possible to recreate acute polymicrobial exposure to life-threatening pathogens through sequential inoculation of mice (Reese et al., 2016). Models that allow for natural

transmission of pathogens would complement these approaches and greatly expand our tool kit for studying host-microbe interactions.

Laboratory mice are typically maintained in “clean” specific pathogen-free (SPF) facilities that seek to reduce experimental variation and unwanted health effects by excluding a list of known transmissible agents. In contrast, “dirty” pet store mice are not subjected to the same level of screening and can harbor multiple pathogens, including disease-causing viruses. Pioneering studies have shown that clean laboratory mice exposed to dirty pet store mice acquire a more developed immune system that serves as a better model for adult humans (Beura et al., 2016; Choi et al., 2019; Huggins et al., 2019). These previous studies focused on the dramatic impact of pathogen exposure on immune function and inflammatory reactions. In this study, Fay et al. modify this approach to study natural transmission of viruses both within and between host species (Fay et al., 2021). The experimental setup consists of cohousing pet store mice with SPF laboratory mice and monitoring for transmission of viruses and other infectious agents. This approach facilitates a complete transmission chain of mouse-specific pathogens through natural routes of infection. Transmission occurs through sustained exposure to physiological levels of pathogens instead of an arbitrary or high-



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concentration inoculum, thereby mimicking real-world infections. Furthermore, this approach can be used to test the role of host pathways in transmission bottlenecks through the use of genetically modified mice, such as IFN knockouts deficient in antiviral immunity.

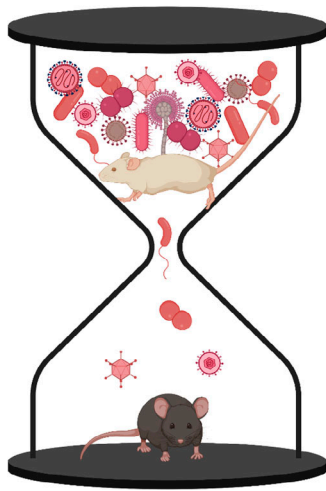
Using this system, the authors identified a multitude of pathogens transmitted to the laboratory mice belonging to at least 10 virus families including novel viruses, most notably a new mouse coronavirus. The data also show transmission of bacteria, fungi, helminths, and protozoans that straddle the line between commensal and opportunistic pathogen. Through analysis of cotransmission, Fay et al. infer relationships between viruses and other microbes that either support or prevent viral transmission. For example, the authors demonstrate that astrovirus infection can reduce a secondary coronavirus infection in an interferon-dependent manner. In addition, there are several co-occurrences or anti-correlations of distinct virus-bacteria pairs

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Cohousing of dirty pet store mice infected with a multitude of pathogens leads to transmission of these agents to clean laboratory mice. Comparing the pathogens in pet store mice (top) with those present in laboratory mice (bottom) through sequencing-based approaches yields insight into infection dynamics, evolution, bottlenecks, and intermicrobe relationships. Swapping pet store mice with pet store rats allows investigation of interspecies transmission. Also, genetically modified laboratory mice can be used to test the role of antiviral pathways in the recipients in preventing transmission. Figure created with BioRender.

that will be of great interest for follow-up studies.

The authors also examine transmission bottlenecks and viral evolution in the new host. For this, they chose astrovirus, one of the most prevalent viruses identified in laboratory mice after cohousing. Amplicon deep sequencing allowed for the analysis of viral variants both in the reservoir as well as the recipient mice. The data shows that while certain viral variants were transmitted to the laboratory mice, others could not be detected. In addition, this experiment also showed that there was a large diversification of astrovirus variants in the new host that was partially driven by antiviral immune responses as it occurred less in certain IFN knockout animals.

Finally, the authors adapted the model to study cross-species transmission between rats and mice. As these two species are in a

predator-prey relationship and cannot be cohoused, soiled bedding and stool from cages housing pet store rats were used to transfer the microbiome and associated pathogens to laboratory mice. Fay et al. (2021) observed transmission of a rat astrovirus with limited replication in mice, indicating a dead-end transmission event. Interestingly, knockout of IFN did not render mice more permissive for rat pathogens, highlighting other barriers to transmission between the two host species. For certain viruses, the process of zoonosis likely involves multiple dead-end infections before random or sequential mutagenesis leads to successful adaptation to the new host. In this context, it is notable that the authors found sequence variants of the rat astrovirus in mice that were not observed in the original rat samples. One can imagine that, given enough opportunities, a bona fide species crossover event will occur in this system, which would be a golden opportunity to examine viral evolution in a safe and controlled laboratory environment.

In conclusion, the authors establish an exciting and timely new model to investigate natural transmission within and between species that will enable investigation into barriers for successful spread of pathogens within a species or for zoonotic introduction into a new host. The initial findings using this new experimental approach raise many questions. For example, how would the different viruses evolve when allowed time to adapt from pet store mice and rats to laboratory mice when using extended transmission chains? What are the differences between transmitting and non-transmitting viruses, and what can this tell us about barriers and bottlenecks of transmission? Other avenues for future research include the use of different reservoirs to explore a wider range of pathogens and other microbiome members, for example different mouse species (here, both reservoir and recipient are *Mus musculus*) or different sources (wild mice or zoo animals). In addition, tapping into one of the biggest advantages of laboratory mice, the availability

of many different knockout mice, could elucidate the role of immunological barriers to virus transmission and within-host evolution. Finally, this study highlighted the potential for extensive interactions between infectious agents that can influence susceptibility to a given viral infection. Detailed knowledge gained from elucidating these interactions may help us identify risk factors for contracting infections or even suggest strategies to curb transmission for human viruses by pitting microbe against microbe.

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