

Embryonated eggs as an alternative infection model to investigate *Aspergillus fumigatus* virulence.

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Abstract

Infection models are essential tools for studying microbial pathogenesis. Murine models are considered the "gold standard" for studying in vivo infections caused by *Aspergillus* species, such as *A. fumigatus*. Recently developed molecular protocols allow rapid construction of high numbers of fungal deletion mutants, and alternative infection models based on cell culture or invertebrates are widely used for screening such mutants to reduce the number of rodents in animal experiments. To bridge the gap between invertebrate models and mice, we have developed an alternative, low-cost, and easy-to-use infection model for *Aspergillus* species based on embryonated eggs. The outcome of infections in the egg model is dose and age dependent and highly reproducible. We show that the age of the embryos affects the susceptibility to *A. fumigatus* and that increased resistance coincides with altered chemokine production after infection. The progress of disease in the model can be monitored by using egg survival and histology. Based on pathological analyses, we hypothesize that invasion of embryonic membranes and blood vessels leads to embryonic death. Defined deletion mutant strains previously shown to be fully virulent or

partially or strongly attenuated in a mouse model of bronchopulmonary aspergillosis showed comparable degrees of attenuation in the egg model. Addition of nutrients restored the reduced virulence of a mutant lacking a biosynthetic gene, and variations of the infectious route can be used to further analyze the role of distinct genes in our model. Our results suggest that embryonated eggs can be a very useful alternative infection model to study *A. fumigatus* virulence and pathogenicity.

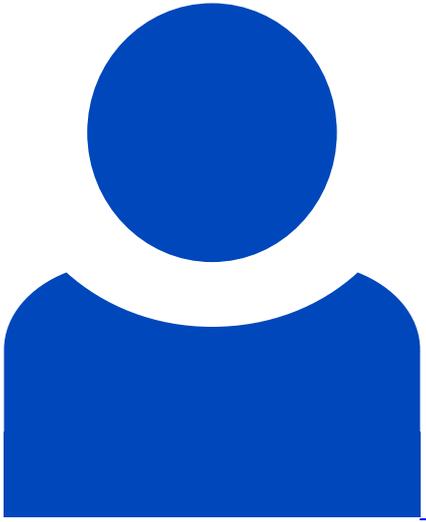
Involved units

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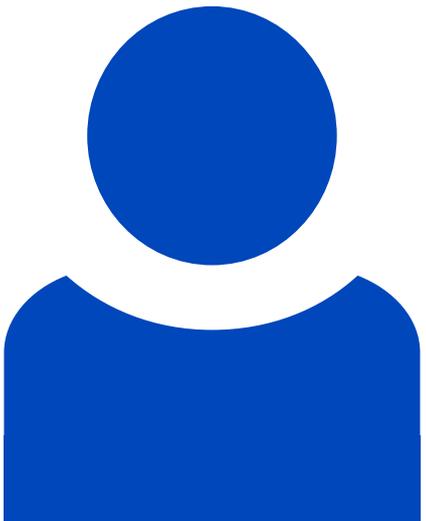
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Leibniz-HKI-Authors



Matthias Brock

[Details](#)



Katharina Große

[Details](#)



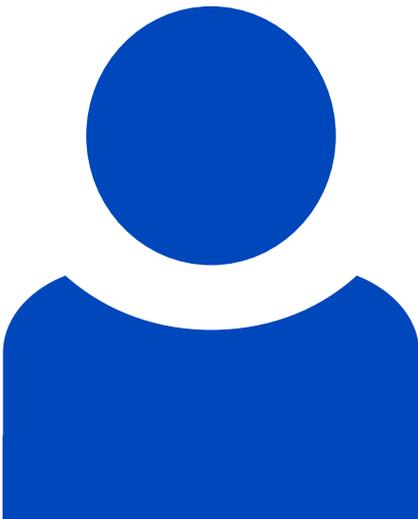
Bernhard Hube

[Details](#)



Ilse Denise Jacobsen

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Silvia Slesiona

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