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Comprehensive pathogen screening of urban rats

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The association between brown rats (*Rattus norvegicus*) - one of the most widespread commensal rodent species - and potentially zoonotic viral, bacterial and protozoan pathogens is well established. This association becomes increasingly important as urban settlements grow and human-wildlife interactions increase. Moreover, changes in resource abundance and distribution by e. g. droughts or food supplementation, as well as resistance to commonly used anticoagulant rodenticides may significantly influence rodent competition and population growth. This, in turn, has implications for the dissemination of pathogens. In order to better predict and manage the threat of zoonotic transmission events, it is necessary to screen rodents for known and novel pathogens, and for rodenticide resistance.
In a proof-of concept study, six rats, collected in Baden-Wuerttemberg, Germany, between May and July 2021, were screened using a combination of pathogen-specific PCR methods (for 35 known pathogens), multiplex RT-PCR/PCR and an open-view method (next-generation sequencing (NGS)). For the NGS analysis, nucleic acid pools of faeces, liver and spleen samples of the six animals were investigated. Pathogen-specific methods were applied on different tissue samples appropriate for each target pathogen. In addition, tail tissues of the rats were analysed for the resistance-mediating VKOR variant tyrosine139cysteine (Y139C) using the amplification refractory system (ARMS)-PCR test.
NGS revealed the presence of zoonotic rat hepatitis E virus (ratHEV), several rat-specific viruses of different taxa and several clinically important bacteria. Pathogen-specific single-plex and multiplex RT-PCR/PCR methods were then used to independently verify the presence of these zoonotic and human pathogenic bacteria (e.g. *Streptobacillus moniliformis*, *Leptospira* spp., *Rickettsia* spp., *Enterobacter cloacae*), zoonotic ratHEV and several rat-specific viruses. The results of these investigations confirmed the outcome of the NGS approach for these pathogens (above). Finally, we have not detected the rodenticide resistance-mediating Y139C polymorphism in the rat samples.
Conventional screening methods typically focus on a single or a small number of pathogens. Consequently, other potentially pathogenic and zoonotic infectious agents may be overlooked. Here, pathogen-specific methods confirmed the presence of infectious agents detected by NGS, providing support for the use of this method in pathogen screening of rodents. Furthermore, this study highlights the diversity of potential pathogens that commensal rodents may harbor and reiterates the importance of regular screening of urban rodents.

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