## Health, zoonotic pathogens and parasites

## Impact of broadleaved hedgerows on the distribution of rodentassociated pathogens and ticks in pine monocultures

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DOI: 10.20315/evmc.2025.090

Understanding the complex relationships between landscape and pathogens distribution in wildlife is crucial for evaluating and mitigating zoonotic risks. Ecological restoration has gained attention as a nature-based solution to bring back biodiversity in human-altered environments. However, the influence of restoration efforts on zoonotic risks remains poorly understood. In Western France, the intensively managed Landes Forest is dominated by maritime pine monoculture (>70%). The plantation of broadleaved hedgerows has been planned to restore forest biodiversity and improve forest resilience. However, the impact of these modifications on zoonotic risks has yet to be assessed.

To investigate whether hedgerows and landscape characteristics (i.e., connectivity and proportion of surrounding broadleaved vegetation) influence pathogen richness and prevalence in rodent populations, we conducted rodent and tick sampling in the Landes Forest during the spring and fall of 2023 and 2024. Our protocol included 53 trapping lines across 7 broadleaved forests, 24 broadleaved hedgerows, and 12 control trap lines along pine plot edges. Orthohantaviruses were detected using serological approaches. *Leptospira* spp. were analysed using a qPCR and the *lipL32* gene. Other pathogenic bacteria were detected with no *a priori* using a metabarcoding approach targeting the V4 region of the 16S-rRNA in the rodent spleen. *Bartonella* sp. identification was later refined using *gltA* and *rpoB* gene primers. Tick specimens were collected individually from rodents and via tick-flagging. Tick species and their pathogens' identification was achieved using a dedicated microfluidic chip.

Wood mouse (*Apodemus sylvaticus*) accounted for 95% of the 380 rodents trapped. Among the 311 wood mice analysed, we did not find any Orthohantavirus-seropositive individuals. We identified 12 putative pathogen bacteria (among which four *Bartonella* species, no *Leptospira* sp.) and three tick species. Notably, mice in area with fewer surrounding broadleaved trees showed a higher likelihood of infection with the tick-borne zoonotic pathogen *Neoehrlichia mikurensis*. Moreover, hedgerows exhibited a higher abundance of ticks than pine edges. These findings underscore the significant influence of landscape modifications on zoonotic hazards. Understanding the mechanisms underlying these relationships—such as shifts in host susceptibility, changes in host-pathogen encounter rates or altered vector distributions—is crucial for integrating effectively zoonotic risk management into ecological restoration practices.

