

CIRCULATION OF ZONOTIC AND PATHOGENIC LEPTOSPIRA IN URBAN RODENTS

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Abstract Leptospirosis is a globally prevalent zoonotic disease caused by pathogenic *Leptospira*, with wild rodents serving as primary reservoirs. This pilot study investigated the presence of *Leptospira* spp. in small mammals from Porto, Portugal. Kidney samples from 23 individuals were analyzed using real-time PCR, *secY* sequencing, and multilocus sequence typing (MLST). *Leptospira* DNA was detected in 13% of the animals, identifying *L. kirschneri* ST117 in *Mus musculus* and *Mus spretus*, and *L. borgpetersenii* ST149 in *Apodemus sylvaticus*. These findings highlight the role of synanthropic rodents as reservoirs of zoonotic *Leptospira*, emphasizing the need for enhanced public health surveillance and risk assessment.

Key words *Leptospira*, zoonosis, rodents

INTRODUCTION

Leptospirosis is a widespread zoonosis caused by pathogenic *Leptospira*. Various animal species can harbor *Leptospira* spp., with wild rodents serving as primary reservoirs (Strand et al., 2023). Transmission occurs through contact with contaminated urine (Strand et al., 2023). In humans, symptoms range from mild flu-like manifestations to severe multiple organ failure (Haake and Levett, 2015), affecting over one million people globally each year and causing approximately 60,000 deaths (Costa et al., 2015).

MATERIALS AND METHODS

Small mammals were captured in the Porto district, Portugal, using Smart boxes that activate upon entry, delivering a fatal electric shock. The system sends a notification to the technician, allowing for prompt collection of the trapped small mammal. Twenty-three kidney samples were obtained, originating from 16 rodents and seven insectivores. Nucleic acid extraction was performed automatically using a commercial kit. For *Leptospira* spp. detection, a real-time PCR

targeting the *LipL32* gene was applied (Stoddard et al., 2009). Positive samples underwent PCR targeting the *secY* gene to identify the *Leptospira* species (Victoria et al., 2008), followed by multilocus sequence typing (MLST) (Boonsilp et al., 2013).

RESULTS

In this pilot study, 13% of the animals tested positive for *Leptospira* spp. Multilocus sequence typing and *secY* sequencing revealed the presence of *L. kirschneri* sequence type (ST) 117 in one *Mus musculus* and one *Mus spretus*, and *L. borgpetersenii* ST149 in one *Apodemus sylvaticus*.

DISCUSSION

This study reports the presence of zoonotic *Leptospira* in various species of urban rodents in Portugal. The detection of pathogenic *Leptospira* in synanthropic rodents underscores their crucial role as pathogen reservoirs with significant public health risks. It also emphasizes the urgent need for continued small mammal monitoring, enhanced public health surveillance and more effective risk assessment strategies.

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REFERENCES CITED

- Boonsilp, S., Thaipadungpanit, J., Amornchai, P., Wuthiekanun, V., Bailey, M.S., Holden, M.T.G., Zhang, C., Jiang, X., Koizumi, N., Taylor, K., Galloway, R., Hoffmaster, A.R., Craig, S., Smythe, L.D., Hartskeerl, R.A., Day, N.P., Chantratita, N., Feil, E.J., Aanensen, D.M., Spratt, B.G., Peacock, S.J., 2013. A single multilocus sequence typing (MLST) scheme for seven pathogenic *Leptospira* species. PLoS Negl. Trop. Dis. 7, e1954.
- Costa, F., Hagan, J.E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M.S., Stein, C., Abela-Ridder, B., Ko, A.I., 2015. Global Morbidity and Mortality of Leptospirosis: A Systematic Review. PLoS Negl. Trop. Dis. 9, e0003898.
- Haake, D.A., Levett, P.N., 2015. Leptospirosis in Humans. Curr. Top. Microbiol. Immunol. 387, 65–97.
- Stoddard, R.A., Gee, J.E., Wilkins, P.P., McCaustland, K., Hoffmaster, A.R., 2009. Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the *LipL32* gene. Diagn. Microbiol. Infect. Dis. 64, 247–255.
- Strand, T.M., Olsson Engvall, E., Lahti, E., Hjertqvist, M., Lundkvist, Å., 2023. *Leptospira* Status in Sweden during the Past Century, Neglected and Re-Emerging? Microorganisms 11, 1991.
- Victoria, B., Ahmed, A., Zuerner, R.L., Ahmed, N., Bulach, D.M., Quinteiro, J., Hartskeerl, R.A., 2008. Conservation of the *S10-spc-a* Locus within Otherwise Highly Plastic Genomes Provides Phylogenetic Insight into the Genus *Leptospira*. PLoS One 3, e2752.