ISWAVLD 2023
International Symposium of the World Association of Veterinary Laboratory Diagnosticians
29 JUNE-1 JULY 2023 Congress Centre Lyon
Towards the veterinary diagnostics of the future

Main topic : Surveillance and control of emerging diseases

Pathogenic Leptospira species in small terrestrial mammals, genetic diversity and ecoepidemiology in Lyon´s urban and periurban parks.

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Introduction
Leptospirosis is a widespread zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira*[1], which is a significant public health problem, with over one million human cases annually[2]. Rodents are considered as the main reservoirs, especially rats, which host mainly the serovar Icterohaemorrhagiae worldwide[3]. Several studies have shown the existence of other reservoirs, such as mice or hedgehogs, which harbor other leptospires serovars[4,5]. However, our knowledge of circulating *Leptospira* strains in reservoirs other than rats in urban environments is limited. In this context, we developed an eco-health approach to assess the zoonotic risk associated with leptospires in urban green spaces, where contacts between human/small mammals and domestic animals are likely. The objectives of this study were to i) evaluate the prevalence of pathogenic *Leptospira* spp. in small terrestrial mammals present in urban parks (Lyon, France) and their spatio-temporal variability, ii) characterize circulating *Leptospira* strains, and iii) document the biotic factors that shape these variations of leptospires prevalence.

Methods
Small terrestrial mammals (*Rodentia* and *Soricomorpha*) were trapped in spring and autumn, between 2020–2022, at two sites in Lyon: one urban park (Tête d'Or) and one periurban park (Lacroix-Laval). Infected animals were detected using *16S rRNA* and *lipL32* qPCR, and the levels of leptospires prevalence were determined. Genotyping of circulating *Leptospira* strains was performed using a combination of molecular methods. Finally, an eco-epidemiological analysis was performed using a binomial generalized linear model to study the influence of animal features (species, age, sex), season, and park on *Leptospira* carriage.

Results
626 animals were analysed, including seven species (*Rattus norvegicus, Mus musculus, Apodemus flavicollis, Apodemus sylvaticus, Myodes glareolus, Microtus arvalis,* and *Crocidura russula*). The global prevalence of leptospires was 10.9%, with variations observed among the seven small mammal species (from 0 to 26.1%). *Rattus norvegicus* showed the highest infection level. Genotyping analyses showed the presence of *L. interrogans*, a genotype related to the Icterohaemorrhagiae serogroup, in rats. Genotypes related to the Australis serogroup were identified in mice, wood mice, yellow-necked mice, and bank voles. *L. kirschneri*, a genotype related to the Grippotyphosa serogroup, was detected in shrews. Lastly, we found that the probability of finding an infected animal was higher in the periurban park than in the urban park (logistic regression, OR = 8.32, 95%CI=2.17-31.87), and in autumn than in spring (logistic regression, OR = 5.57, 95%CI=1.59-19.56).

Conclusions
Our study showed a significant carriage of leptospires in small terrestrial mammals in Lyon city, with strong variations observed between animal species. *Leptospira* zoonotic risk seemed to be higher in the periurban park and in autumn, potentially due to forest connectivity and small mammal communities. Our study suggested an important public health relevance of rats and urban adapter rodents (*Apodemus spp.* and *M. glareolus*) as reservoirs of *L. interrogans*. Further investigations with an increased number of parks are needed to better understand the impact of urbanization, and the evolution of the prevalence of leptospires over time.