



Characterisation of pathogenic *Leptospira* in invasive raccoons (*Procyon lotor*) in northeast and southwest France

Short Paper

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Corresponding author:

Florence Ayrál;

Email: florence.ayral@vetagro-sup.fr

[†]These authors contributed equally to this work

Florence Ayrál^{1†} , Elena Harran^{1†}, Christine Fournier-Chambrillon², Manon Gautrelet^{2,3,4}, Océane Tourniaire¹, Alexis Peutot¹, Karine Groud¹, Nathan Thenon^{2,5}, Pascal Fournier² and Céline Richomme⁶

¹USC 1233-RS2GP, VetAgro Sup, Université de Lyon, Marcy L'Etoile, France; ²Groupe de Recherche et d'Etude pour la Gestion de l'Environnement (GREGE), Villandraut, France; ³Centre de Recherche et de Formation en Eco-éthologie (CERFE), Université de Reims Champagne-Ardenne, Boult-aux-Bois, France; ⁴Groupe d'Etude sur les Géomatériaux et Environnements Naturels, Anthropiques et Archéologiques (GEGENAA), Université de Reims Champagne-Ardenne, Reims, France; ⁵UMR 1225, INRAE, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France and ⁶Anses, Nancy Laboratory for Rabies and Wildlife, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Malzéville, France

Abstract

Leptospirosis is a widespread zoonosis caused by bacteria of the genus *Leptospira*. Although crucial to mitigate the disease risk, basic epidemiological information is lacking, such as the identities of *Leptospira* maintenance hosts. The raccoon (*Procyon lotor*), an alien invasive species in France, could pose a public health risk if it carries pathogenic *Leptospira*. We investigated the rate and type (selective vs. unselective) of *Leptospira* carriage in the two main raccoon populations in France. Out of the 141 raccoons collected, seven (5%) tested quantitative PCR positive, targeting *lfb1* gene, based on kidney, lung, and urine samples. Phylogenetic analysis revealed the presence of three different *L. interrogans* clusters. The results suggest that raccoons were more likely accidental hosts and made only a limited contribution to *Leptospira* maintenance.

Leptospirosis is a global and potentially fatal zoonotic disease that affects all mammals, including humans, and is caused by pathogenic species of the genus *Leptospira*. The genetic polymorphism diversity of eight pathogenic species was recently studied using the *lfb1* gene, identifying 46 species groups [1]. *Leptospira* is mainly transmitted through soil or water contaminated by the urine of infected animals. The bacteria can survive for days in aquatic environments, which are the origin of most human cases of leptospirosis. However, the source of environmental contamination is a range of mammalian maintenance hosts: *Leptospira* colonizes the kidneys, where they remain over the long term and are shed in the urine [2].

Although leptospirosis is a major public health burden, management strategies are limited due to a lack of basic epidemiological knowledge, such as the role of various animal hosts in *Leptospira* maintenance across ecosystems. In order to assess the ability of a given mammal species to maintain *Leptospira* and to design better disease prevention approaches, it is crucial to determine the prevalence of *Leptospira* in target animal populations, as well as any host–pathogen adaptations that may be present (i.e., whether the target animals exclusively carry a particular *Leptospira* strain) [2]. The raccoon (*Procyon lotor*) is a North American species that has become invasive worldwide, notably in Europe and different regions of mainland France [3]. *Leptospira* may circulate endemically in raccoons in North America or sporadically, as previously suggested in France [4, 5]. Therefore, the raccoons make different contributions to *Leptospira* epidemiology across ecosystems and it is important to explore the potential variability within populations in the country.

The aim of this study was to assess *Leptospira* infections in the two main raccoon populations in France. In addition, we aimed to determine whether selective carriage occurs in raccoons by genetically characterising any *Leptospira* DNA retrieved.

This work used a sub-sample of a wider study related to raccoon's ecology, in northeastern and southwestern France, between 2019 and 2021 [6]. The raccoons were found dead on the roads or trapped by duly licensed trappers in the context of invasive population management and sacrificed in accordance with the regulations on alien invasive species (French decree of 2 September 2016) and animal welfare guidelines (Directive 2010/63/EU). Therefore, an application to the Ethics Committee was not required.

All the animals were frozen immediately after collection. They were later thawed for necropsy to obtain kidney, lung, and urine samples (if available) from each animal. Kidney tissue and urine are the preferred biological materials for the detection of *Leptospira*. The lung was also included

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example, rats are the primary hosts for the *L. interrogans* serogroup Icterohaemorrhagiae [2]. The diversity of *Leptospira* species-groups we found in raccoons supports the idea that carriage is not selective, as has been suggested elsewhere [5, 12]. In other words, raccoons may be sporadically infected by strains present in the environment but not able to maintain particular strain for prolonged periods. However, only one sample (RLG57R) coming from the southwest population could be typed. Additional raccoons should be therefore analysed to further clarify the species group diversity in this subpopulation.

Lastly, the magnitude of the *Leptospira* infection rate was similar in raccoons sampled in northeastern and southwestern France, suggesting that raccoons could have similar epidemiological contributions in both regions and ecosystems.

In conclusion, our results suggest that, unlike rats, raccoons are unlikely to maintain *Leptospira*, although they may spread them somewhat in the environment. However, some raccoon populations are currently found in peri-urban areas, in close proximity to humans and dogs. The risk of transmission should not be neglected, especially if the raccoon densities increase. Under such conditions, it would be important to reassess the risk of *Leptospira* transmission associated with raccoons.

Data availability statement. The data that support the findings of this study are available on request.

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