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

Florence Ayral[1†], Elena Harran[1†], Christine Fournier-Chambrillon[2], Manon Gautrelet[2,3,4], Océane Tourniaire[1], Alexis Peutot[1], Karine Groud[1], Nathan Thenon[2,5], Pascal Fournier[2] and Céline Richomme[6]

[1]USC 1233-RS2GP, VetAgro Sup, Université de Lyon, Marcy L'Etoile, France; [2]Groupe de Recherche et d'Etude pour la Gestion de l'Environnement (GREGE), Villandraut, France; [3]Centre de Recherche et de Formation en Eco-éthologie (CERFE), Université de Reims Champagne-Ardenne, Boult-aux-Bois, France; [4]Groupe d'Etude sur les Géomatériaux et Environnements Naturels, Anthropiques et Archéologiques (GEGENAA), Université de Reims Champagne-Ardenne, Reims, France; [5]UMR 1225, INRAE, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France and [6]Anses, 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.

Short Paper

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Corresponding author:
Florence Ayral;
Email: florence.ayral@vetagro-sup.fr

[†]These authors contributed equally to this work

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

because a previous study showed lung colonisation in rats [7]. The samples were then stored at –20°C until further analysis. DNA extraction was performed using the Nucleospin Tissue Kit (Macherey Nagel, Hoerd, France) according to the manufacturer's instructions, and DNA samples were stored at –20°C until the molecular analyses could be performed. The presence of pathogenic *Leptospira* DNA in the kidney, lung, and urine samples was assessed by quantitative PCR (qPCR) targeting the 16S rRNA (*rrs*) gene and the AgPath-ID™ One-Step qPCR Reagents (Applied Biosystems, Austin, United States), as described elsewhere [8]. DNA samples with a cycle threshold (Ct) of less than 40 were considered to be positive and were further amplified by conventional PCR (cPCR) targeting the *lfb1* gene as described previously [9]. The amplified products were verified by 1% agarose gel electrophoresis and subjected to Sanger sequencing (Genoscreen, Lille, France). Sequencing of the *lfb1* gene provides information on the species group, which is related to both the bacterial species and the genogroup [1]. This approach could increase typing success when working with wildlife samples that may contain damaged DNA or low quantity of *Leptospira* DNA. A nucleotide BLAST search was performed (NCBI: http://blast.ncbi.nlm.nih.gov) to identify the *Leptospira* species. A phylogenetic tree was then generated using the *Leptospira* spp. *lfb1* partial gene polymorphism in raccoon samples and reference strains provided elsewhere [1].

We included a total of 141 raccoons (100 and 41 from northeastern and southwestern France, respectively). Seven raccoons were qPCR positive based on kidney and/or urine samples. All the lung samples were qPCR negative. Among the tested raccoons, seven were infected (5%, $CI_{95\%}$ [2%, 10%]), including five from northeastern France and two from southwestern France. *L. interrogans* was detected in six samples coming from five of the seven qPCR-positive raccoons (two *Leptospira* typing failed). Phylogenetic analysis identified three distinct *lfb1* species groups: the first (three amplicons coming from two raccoons) was described in reference strains belonging to the Autumnalis, Bataviae, and Australis serogroups; the second (one amplicon) was described in a reference strain belonging to the Djasiman serogroup; and the third (two amplicons) was described in reference strains belonging to the Icterohaemorrhagiae serogroup (Figure 1).

Our results suggest that raccoons could potentially spread pathogenic *Leptospira* given that *Leptospira* DNA was found in the kidney and urine samples. However, raccoons seem more likely to serve as non-maintenance or accidental hosts (i.e., short-term infection and shedding) than as maintenance hosts (i.e., long-term infection and shedding). Indeed, maintenance hosts, namely *Rattus* species, have prevalence levels exceeding 20% in France [11] and in other countries as a result of chronic renal colonisation [2]. Populations with lower prevalence levels are therefore unlikely to maintain *Leptospira* for long periods. In this study, the *Leptospira* infection rate encompassed 5% ($CI_{95\%}$ [2%, 10%]) of the tested raccoons, which is lower than that found in rats, and this magnitude was strengthened by a recent study in Germany, a bordering country [12]. In contrast to rats, *Leptospira* DNA was not detected in the lung, highlighting possible differences in the pathogeny or host adaptation between the two species.

In addition, we identified three species groups of *Leptospira* within the infected raccoons, a result that is consistent with the hypothesis that they are non-maintenance hosts. Indeed, *Leptospira* maintenance hosts appear to selectively carry specific strains; for

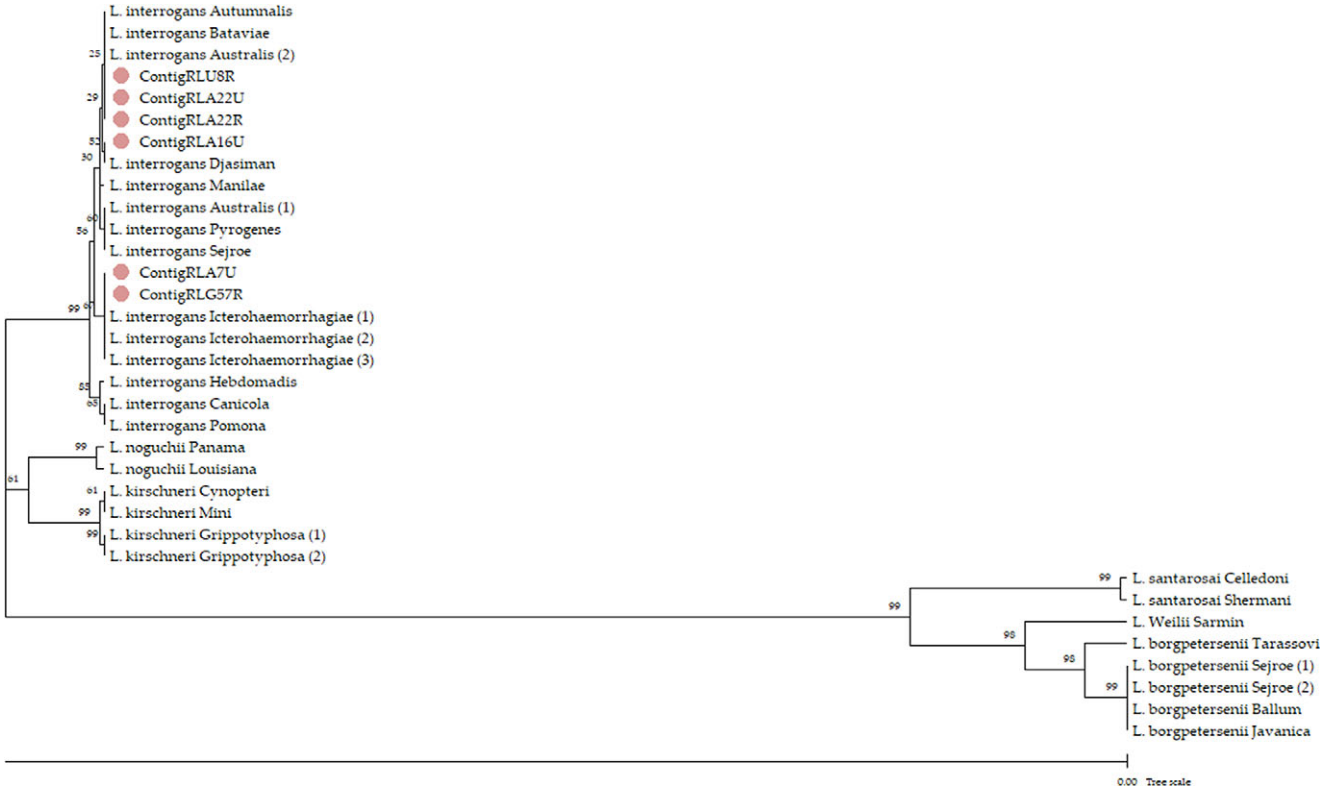

Figure 1. Phylogenetic tree of *Leptospira* DNA extracted from raccoons and characterized based on the *lfb1* gene sequences (bootstrap analysis involving 1 000 replicates and 34 nucleotide sequences). The evolutionary distances (unit = base substitutions per site) were computed using the maximum composite likelihood method [10] and MEGA11 software. The pink circles represent the amplicons from the samples. The contigs are identified using a coding system with the format 'RL NN M': where NN indicates sample geographical origin (northeastern France = A and U, southwestern France = G) plus ID number and M indicates sample tissue type (urine = U, kidney = R).

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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