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Tracking potential *Leptospira* sources following human cases of leptospirosis: A One Health approach applied to an ecosystem in Brittany, France

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ABSTRACT

Pathogenic *Leptospira* can cause leptospirosis: a widespread, potentially fatal bacterial zoonosis whose risk is mediated by the soil and water features, animal host distributions, meaning the local ecosystem. When human cases of leptospirosis occur, it is challenging to track down their source because ecosystem-level epidemiological knowledge on *Leptospira* is needed. Between 2016 and 2019 in a focal riparian ecosystem, the human population experienced an outbreak and successive cases of leptospirosis attributable to L. *kirschneri* and L. *interrogans*. The epidemiological investigation was carried out using the One Health approach, as described in international health guidelines. As a first step in this process, we investigated leptospiral carriage in the main animal hosts found in the region. We sampled 143 nutrias, 17 muskrats, and 10 Norway rats using convenient trapping. DNA was extracted from their kidneys, lungs, and urine and subjected to real-time PCR (RT-PCR) targeting the *Leptospira* 16S rDNA and *lfb1* genes. In the farms along the river's stretch of interest, we sampled serum from 439 cattle and used a microscopic agglutination test to detect the presence of antibodies against *Leptospira*. Urine samples were concomitantly obtained from 145 cattle and were used in two analyses: RT-PCR targeting the *Leptospira* 16S rDNA gene and *Leptospira* culturing. We found th, wt rodents were the most likely source of the L. *interrogans* behind the human cases. The cattle tested negative for *Leptospira* DNA but positive for antibodies against the serogroups implicated in the human cases. We failed to identify the potential source of the L. *kirschneri* responsible for several human cases of leptospirosis. Our results call for further clarification of the *Leptospira* maintenance community, which may comprise known maintenance hosts, such as rodents, as well as taxa not commonly considered to be maintenance hosts but that can still spread *Leptospira*. The resulting research

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network will collaboratively conduct future eco-epidemiological surveys to illuminate the leptospirosis risks faced by humans and animals within ecosystems.

1. Introduction

Leptospirosis is a widespread bacterial zoonosis whose occurrence is strongly influenced by ecohydrological dynamics, giving rise to endemic and epidemic patterns of infection [1]. The disease can result in systemic organ failure and death in both animals and humans [2]. The genus *Leptospira* is currently subdivided into 69 genomic species, including saprophytic or pathogenic bacteria [3,4]. Infections in humans and animals are caused by eight pathogenic species [3], which are divided up into at least 26 serogroups [5]. Annually, members of the genus, predominantly L. *kirschneri* and L. *interrogans*, cause an estimated 1 million cases of disease and 60,000 deaths in human populations worldwide [6]. Several leptospirosis outbreaks in Western Europe have been traced back to L. *kirschneri* [7–9], and both bacterial species commonly occur in wild and domestic animals [10–13].

Humans mainly become infected through exposure to soil or water contaminated by the urine of infected animals [2]. Water sports and occupational activities have been identified as risk factors, but outbreak occurrence remains otherwise unpredictable across space and time [14]. Over the last two decades, Western Europe has experienced leptospirosis outbreaks related to water sports, but the animal sources potentially responsible were not described [8,15] or described with weak supporting evidences [16]. While all mammals can be infected by pathogenic *Leptospira*, a given host's relative contribution to environmental contamination depends on the host-pathogen relationship as well as on how animal populations interact with each other and the environment. Thus, the specific reservoirs causing leptospirosis infections in humans depend on ecosystem characteristics, which makes it challenging to implement standardized preventive measures [17]. Water sports typically take place in ecosystems containing high levels of mammalian biodiversity and, therefore, a variety of potential *Leptospira* hosts, which contrasts with the low-diversity situation in urban ecosystems, where murines usually serve as reservoir hosts [18,19]. Several mammals can serve as accidental or maintenance hosts for different serovars [20]. Consequently, the sum of an ecosystem's *Leptospira* hosts can be thought of as a maintenance community, promoting environmental contamination and causing *Leptospira* infections in other animals and humans [21]. It is thus crucial to identify the potential hosts that make up maintenance communities to comprehensively assess human and animal health risks.

In western Europe, with the climate change and rise in mean temperature, aquatic recreational activities is expected to increase and there may be variation in the dynamics of wild animal populations [22]. In addition, the changes in patterns of precipitation and in mean temperature could foster *Leptospira* survival for longer in the environment [23,24]. Consequently, we are likely to see more leptospirosis outbreaks in the coming decades [25]. Ecoepidemiological surveys implemented according to a collaborative multisectorial and disciplinary approach are needed if we wish to fully characterize the patterns of leptospirosis outbreaks. Although international organizations recommend such an approach, namely the One Health approach [14,26,27], there remains a lack of local-level frameworks and examples for putting One Health into practice.

As a first step, we investigated *Leptospira* carriage in various animal populations found in an ecosystem where a leptospirosis outbreak occurred among kayakers in September 2016 ($n = 14$ cases, of which 8 were confirmed by serological tests or polymerase chain reaction [PCR]). Additional human cases of leptospirosis appeared in May–June 2018 ($n = 3$ confirmed clustered cases and 5 probable cases) and in November 2019 (n = 1 confirmed case and 1 probable case). In 8 of the 12 confirmed cases, typing or serological profiling identified the causative bacteria as L. *kirschneri* (n = 3), *L. interrogans* (n = 1), or a member of the serogroup Grippotyphosa ($n = 4$) [8,28], to which both L. *kirschneri* and L. *interrogans* belong [29].

In this study, our goal was two-fold. First, we sought to clarify *Leptospira* ecoepidemiology within a focal ecosystem in Brittany that has given rise to recurrent human cases of leptospirosis. Second, we described the framework of *Leptospira* source tracing according to a One Health approach, as described in international health guidelines [30,31], its drawbacks and benefits.

2. Materials and methods

2.1. Ethics statement regarding animal sampling

Samples were collected from cattle in accordance with the procedure approved on September 19, 2018, by VetAgro Sup Ethics Committee (agreement n°1813). The rodents sampled in the study were killed during pest control campaigns by professionals in the field and certified trappers. The pest control campaigns use strategies that adhere to French and European legislation on the treatment and usage of animals (Directive 2010/63/EC and French Administrative Decision 2007/04/06). As a result, the ethics committee considered that its approval was not required.

2.2. Cattle sampling

Ten cattle farms occur along a stretch of the Vilaine River located upstream from Rennes, Brittany's capital city. Eight of these farms (six dairy and two beef) made their herds available to us. The first sampling session took place from March to May in 2019, which was the pre-grazing period; both serum and urine were collected. The second session took place from November 2019 to January 2020, which was the post-grazing period; only serum was collected. Approximately 30 adult cattle per farm were sampled over the course of two sessions. For the second session, cattle sampled in the first session were sampled when possible. Sampling was performed by the farmers' veterinarians and a local farmer's association for herd disease control (*Groupement de Défense Sanitaire*). To collect the urine sample, an animal's vulva was cleaned and disinfected before introducing a catheter directly into the bladder *via* the urethral canal. Samples were also collected when spontaneous urination occurred. Fresh urine specimens were used in our attempt to culture *Leptospira* and were kept at 16–22 °C pending further analysis. The serum samples and other urine samples were stored at −20 °C until the molecular and serological analyses could be performed.

2.3. Rodent sampling

Three rodent species—Norway rats (*Rattus norvegicus*), nutrias (*Myocastor coypus*), and muskrats (*Ondatra zibethicus*)—are all likely excreting leptospires into the Vilaine River. They are also the predominant mammals in the region (survey conducted on July 31, 2018; Supplemental Data 1). Representatives of each species were gathered between March and November 2019, during pest control campaigns conducted in the proximity of two nautical bases (sites A and B, respectively). These areas are located 8 km apart and near where human cases of leptospirosis were reported in 2016 and 2018. The animals' bodies were stored at −20 °C until necropsy and tissue sampling could occur. At that time, any macroscopic abnormality of the thoracic and abdominal organs was sought, samples were taken of the kidneys, lungs, and urine (when available) and subsequently stored at −20 °C until the molecular analyses were conducted. Furthermore, individuals were classified as male (presence of testes) or female (presence of a genital

tract) and as sexually immature (absence of seminal vesicles for males and a developed uterus for females) or sexually mature (presence of these structures).

2.4. Urine cultures

We attempted to culture leptospires from the cattle urine samples using Ellinghausen–McCullough–Johnson–Harris (EJMH) medium and EJMH STAFF medium, which were prepared under sterile conditions as described elsewhere [32]. Briefly, 0.1 ml of urine was added to a first tube containing 5 ml of EJMH STAFF medium. Next, 0.1 ml of this dilution was transferred to a second tube containing 5 ml of EJMH medium, yielding a further dilution (1:50). This second set of tubes was incubated at 30 °C over a two-month period. Their contents were regularly examined using dark-field microscopy to ascertain whether leptospires were present [33].

2.5. DNA extraction and Leptospira detection

DNA was isolated from rodent kidney and lung tissue and from rodent and cattle urine using the QIAamp DNA Mini Kit (Qiagen), in accordance with the manufacturer's instructions. To detect *Leptospira* DNA, we performed real-time PCR (rt-PCR; TaqMan method). We employed the β-actin endogenous housekeeping gene as an internal control for target gene expression, DNA extraction efficiency, and the absence of inhibitors in the samples [34]. We specifically targeted pathogenic *Leptospira* using the TaqVet PathoLept Kit (LSI, France). Samples with C_T values of <40 were considered to be *Leptospira* positive, and a given animal was considered to be infected with *Leptospira* if at least one of its tissues or fluid (kidney, lung, or urine) tested positive.

2.6. Genotyping

Positive samples were then subject to conventional PCR (cPCR). One analysis targeted the 16S rDNA gene (as described elsewhere: [35] and used a DreamTaq Green PCR Master Mix (2×) Kit (Thermo Fisher Scientific) in accordance with the manufacturer's instructions. A second analysis targeted the *lfb1* gene (as described elsewhere: [36] and used a HotStarTaq DNA Polymerase Kit (Qiagen, Courtaboeuf, France) in accordance with the manufacturer's instructions. The amplified products were visualized using electrophoresis (1% agarose gel). Then, samples yielding clearly visible, high-intensity bands underwent Sanger sequencing, which was carried out by a service provider (Genoscreen, Lille, France). The *Leptospira* species present were identified through a Nucleotide BLAST search (http://blast.ncbi.nlm.nih.gov).

2.7. Microagglutination tests

A microscopic agglutination test (MAT) was performed using a panel of live isolated leptospires. They represented 12 serogroups (serovars in parentheses): Australis (Australis, Bratislava, Munchen), Autumnalis (Autumnalis, Bim), Ballum (Castellonis), Bataviae (Bataviae), Canicola (Canicola), Grippotyphosa (Grippotyphosa, Vanderhoedoni), Icterohaemorrhagiae (Icterohaemorrhagiae, Copenhageni), Panama (Panama, Mangus), Pomona (Pomona, Mozdok), Pyrogenes (Pyrogenes), Sejroe (Sejroe, Saxkoebing, Hardjo, Wolffi), and Tarassovi (Tarassovi). A titer of 1:100 was used as the cut-off threshold for seropositivity, as per the guidelines issued by the World Organization for Animal Health (WOAH) [37]. Particular care was taken when interpreting cross-reactions [29,38]. A serogroup-level analysis of the MAT results was performed as described elsewhere [39,40]. When a high titer against a particular serogroup was observed, that single serogroup was assumed to be responsible for the antibodies in the sample. When we obtained titers against several serovars from the same serogroup, the serovar with the highest titer was assigned to the serogroup. When a sample displayed titers against two or more serogroups, we used the following approach. If one serogroup clearly predominated (*i.e.,* its titers were at least threefold higher than those of the next most prominent serogroup), then this serogroup was assigned to the sample. If the difference in titers among serogroups was not as clear cut (*i.e.,* there was less than a threefold difference between the highest titer and the next highest titer), then both serogroups were assigned to the sample as they were equally likely to be the predominant serogroup.

2.8. Statistical analysis

The data were analyzed in Rstudio (v. 1.3.1093, Apricot Nasturtium). The function *binom.test* was used to estimate *Leptospira* prevalence and its 95% confidence intervals. For the nutrias, univariate Poisson regression models were used to examine the factors influencing infection status; the independent variables were sex (male *vs* female), sexual maturity (immature *vs* mature), sampling site (A *vs* B), and sampling season (spring = March to May, summer = June to August, and autumn = September to November). An α-level of 0.05 was used.

2.9. Mapping

We mapped the spatial distributions of the animals sampled, their infection status, and their exposure status for sites A and B using R Core Team (v.4.2.1). The background map came from IGN GEOFLA®.

3. Results

3.1. Leptospira exposure in cattle

The MAT results are summarized in Fig. 1. In the first sampling session, 11 of the 212 serum samples were MAT positive and came from 5 of the 8 farms where sampling occurred. The serological profiles showed that cattle on 4 farms had been exposed to the serogroup Grippotyphosa and cattle on 1 farm had been exposed to the serogroup Sejroe. In the second sampling session, 27 of the 227 serum samples were MAT positive and came from all 8 farms. The serological profiles showed that cattle on 3 additional farms (4, 6, and 7) had been exposed to the serogroup Grippotyphosa and that cattle on 6 farms had been exposed to the serogroup Icterohaemorrhagiae. The latter had not been detected during session 1. Sampling took place at each farm across both sessions, and repeated measures were obtained for 5 to 17 cattle per farm (the only exception being farm 3). These data showed that at least one animal had seroconverted (*i.e.,* was negative during session 1 and positive during session 2). Lastly, neither live *Leptospira* nor *Leptospira* DNA was detected in the urine samples ($n = 145$). While the cultures were positive for other bacterial groups (*i.e.,* sample bacterial contamination), they were negative for *Leptospira,* even when the cattle showed other evidence of *Leptospira* exposure.

Additional details on the MAT, PCR, and culture results are available in Supplemental Data 2.

3.2. Demographic characteristics of the nutrias, muskrats, and Norway rats

The demographic characteristics of the nutrias, muskrats, and Norway rats collected during each sampling season are shown in Fig. 2.A. The total sample size was 170 rodents, which was made up of 143 nutrias, 17 muskrats, and 10 Norway rats. Overall, there were more males than females for the nutrias (males: 59%, $n = 84/143$; females: 41%, $n = 59/143$) and for the muskrats (males: 76%, $n = 13/17$; females: 24%, $n = 4/17$). Additionally, there were more sexually mature nutrias than sexually immature nutrias (sexually mature: 63%, $n = 91/143$, sexually immature: 37%, $n = 52/143$) and muskrats (sexually mature: 59%, $n = 10/17$; sexually immature: 41%, $n = 7/17$). For the Norway rats, the two ratios were equal. Sample sizes varied across seasons. No Norway rats were captured in the spring, one muskrat was captured in the summer,

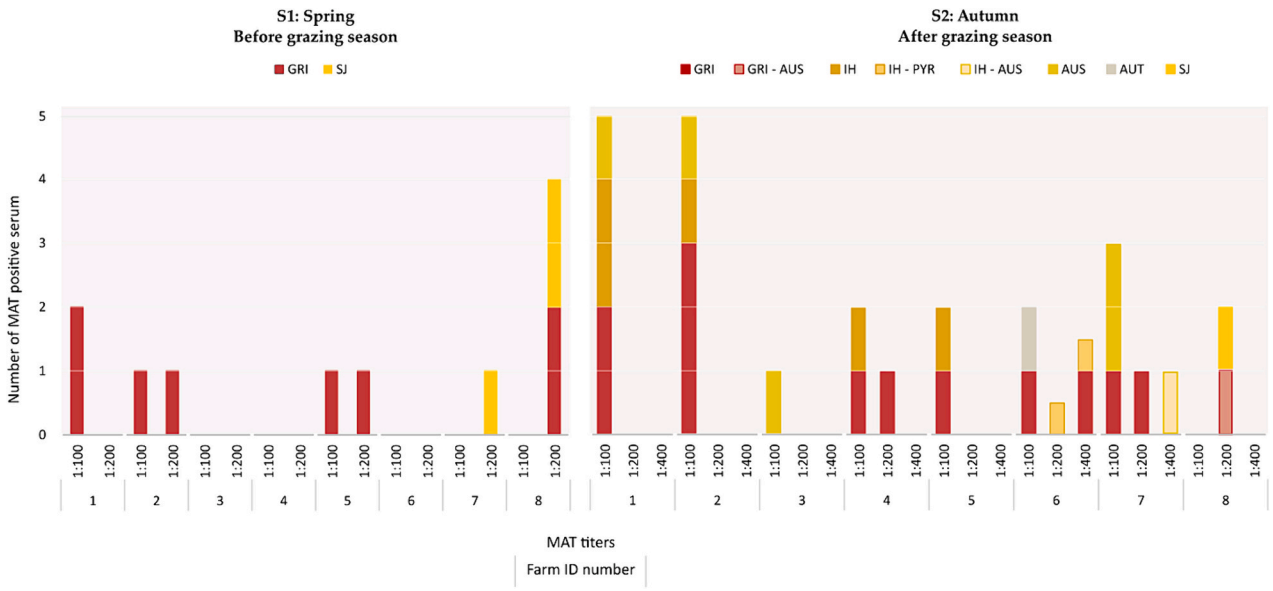

Fig. 1. Number of MAT-positive cattle in session 1 (S1) and session 2 (S2) across the 8 farms, ranked according to titer (1:100 to 1:200 or 1:400).
The different putative serogroups are color coded. The mixed serogroups GRI-AUS and IH-AUS are cases in which the serological profile showed equal titers for two serogroups. On Farm number 6 during session 2, one cattle displayed titers against IH (1:200) and PYR (1:400), which resulted in the mixed serogroups IH-PYR inclusion in the two titer categories.
Abbreviations. GRI: Grippotyphosa; SJ: Sejroe; AUS: Australis; PYR: Pyrogenes; AUT: Autumnalis; IH: Icterohaemorrhagiae

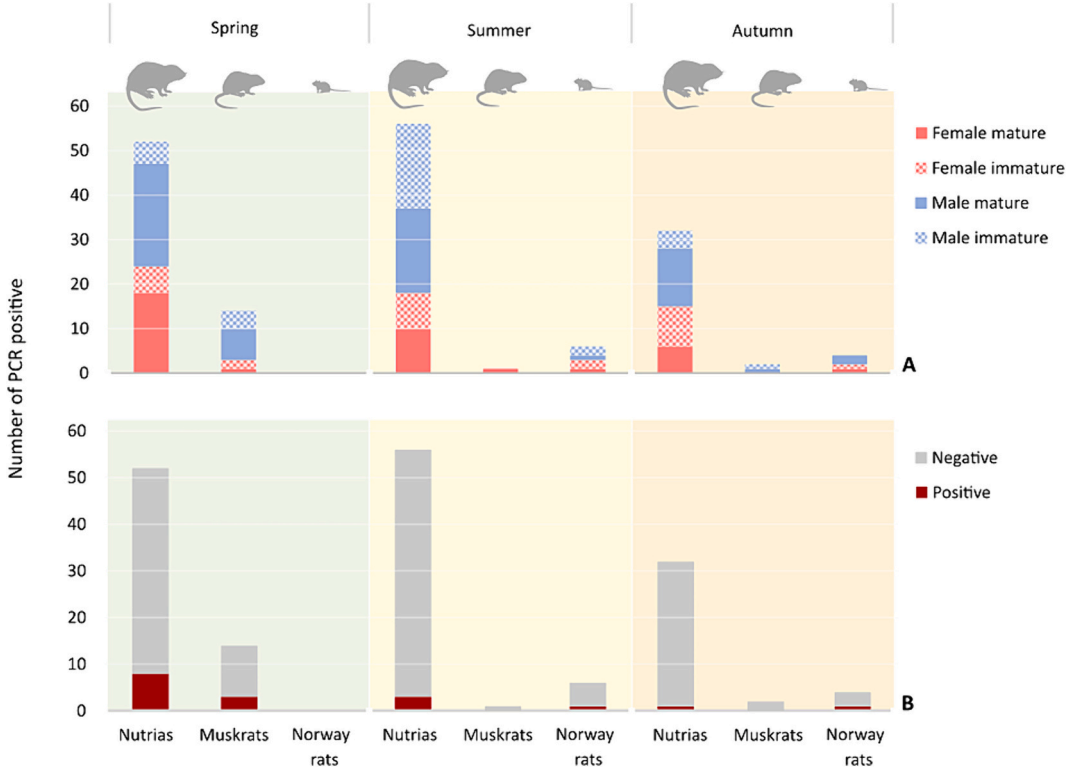

Fig. 2. (A) Demographics for nutrias, muskrats and Norway rats across spring, summer and autumn. The season in which three nutrias were collected (one sexually mature female, one sexually immature female, and one sexually mature male) was not recorded and therefore does not appear above. (B) Number of nutrias, muskrats and Norway rats testing rt-PCR positive and negative across spring, summer and autumn. The three nutrias not shown were rt-PCR negative.

and fewer nutrias were collected in the autumn (n = 36) than in the spring (n = 52) or summer (n = 56). Males outnumbered females in all three seasons.

3.3. *Leptospira infection in the nutria, muskrats, and Norway rats*

3.3.1. *Presence of Leptospira DNA*

Of the 170 rodents collected, 12 nutrias, 3 muskrats, and 2 Norway rats were PCR positive for *Leptospira*. Despite these results, no

morphological abnormalities were seen in the animals' tissues upon dissection. The seasonal pattern in the PCR results is displayed in Fig. 2. B.

Prevalence varied between sites A and B and across rodent species, although not significantly so. The global prevalence was 18% in muskrats (n = 3/17, $CI_{95\%}$ [4%, 43%]), 20% in Norway rats (n = 2/10, $CI_{95\%}$ [2%, 56%]), and 8% in nutrias (n = 12/143, $CI_{95\%}$ [4%, 14%]).

3.3.2. Characterization of Leptospira DNA

For 11 of the 17 PCR-positive rodents, a high-intensity band emerged during cPCR. Subsequent sequencing revealed the presence of L. *interrogans* in 7 nutrias (n = 7/12), 2 muskrats (n = 2/3), and 2 Norway rat (n = 2/2).

3.3.3. Characteristics of the infected rodents

For the nutrias, *Leptospira* prevalence was uninfluenced by sex, sexual maturity, site, or season (p-value >0.05). That said, there was a marginally insignificant trend (p = 0.1) whereby *Leptospira* prevalence seemed to be higher in the spring than in the autumn (PR = 4.9, $CI_{95\%}$ [0.9, 91]) and in males *versus* females (PR = 3.4, $CI_{95\%}$ [0.9, 22]).

3.4. Spatial distribution of rodents and cattle farms

The individuals testing positive for *Leptospira* (*via* PCR or MAT) occurred all along the Vilaine River and across both sites (Fig. 3).

4. Discussion

Our results revealed that substantial numbers of rodents potentially shed L. *interrogans* into the environment across seasons, while the cattle appeared to be experiencing incidental infections, similar to humans. However, our investigation failed to identify the potential source of L. *kirschneri*, which has also caused human cases of leptospirosis. Below, we discuss the potential animal sources of pathogenic *Leptospira* and the challenges inherent to comprehensively describing the reservoirs responsible for human cases of leptospirosis. We also propose a partial multisectorial framework for implementing future eco-epidemiological surveys following cases of leptospirosis associated with aquatic recreational activities.

4.1. Potential animal sources of Leptospira

4.1.1. Cattle were unlikely to be a source of pathogenic Leptospira

Despite our meticulous sampling procedure on the farms, no *Leptospira* were cultured from any of the 145 urine samples and medium were contaminated. This outcome might have been the result of culture contamination, which could have limited our ability to detect leptospires *via* microscopic observations, and/or the result of culture sensitivity, since the technique is far less sensitive than PCR, as highlighted by numerous studies [41,42]. However, the urine samples were also PCR negative for *Leptospira* DNA, confirming the taxon's absence. Taken together, these results strongly suggest that none of the 145 cattle sampled were infected at the time of sampling, which implies that the cattle were not contributing to environmental contamination. In addition, the serological results indicate that the cattle were exposed to the serogroups Grippotyphosa and Icterohaemorrhagiae. These serogroups are rarely seen in cattle from France [39], which suggests an unusual epidemiological situation underlies their circulation at both sites.

4.1.2. Rodents were likely a source of pathogenic Leptospira

The pathogenic species L. *interrogans* was detected in nutrias, muskrats, and Norway rats at the two sampling sites along the Vilaine River, suggesting that infection with this pathogen is common among local rodent populations.

Norway rat. Although few Norway rats were captured due to their usual neophobia and the weather conditions (*i.e.*, persistent flooding) and our estimates of prevalence were imprecise (20%, $CI_{95\%}$ [2%, 56%]), the level of prevalence within the population was comparable to that within other populations in previous studies [43,44]. In addition, the Norway rats were carrying L. *interrogans*, which was not unexpected, given that they are maintenance hosts for L. *interrogans* serogroup Icterohaemorrhagiae [45]. However, we were unable to genetically

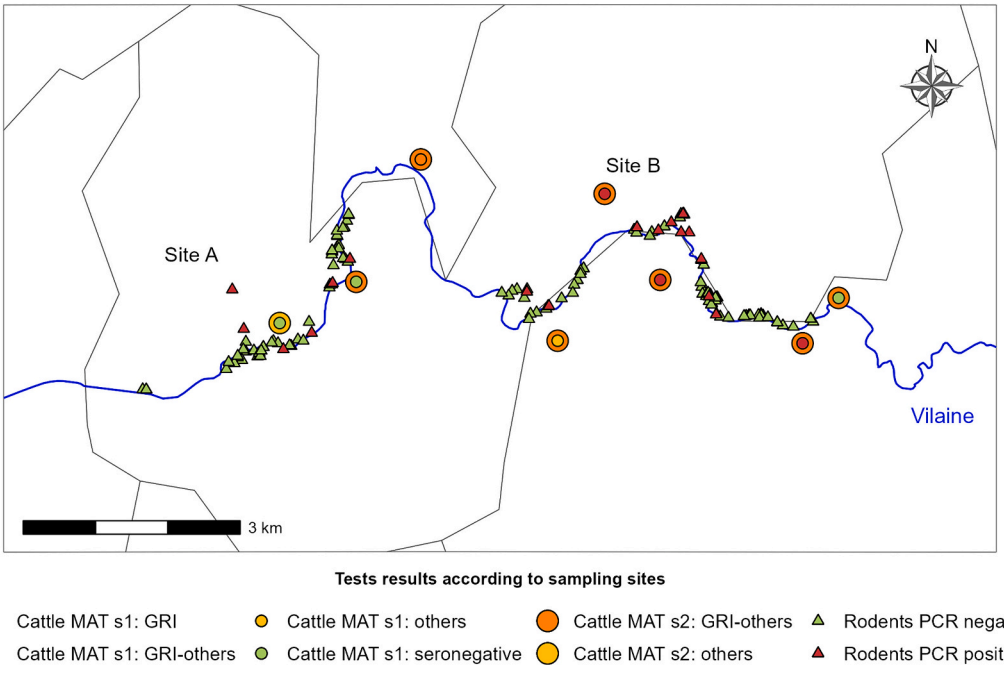

Fig. 3. Spatial distribution of the rodents and cattle farms tested for *Leptospira*. For the cattle, samples were collected during session 1 (S1) and session 2 (S2). There were farms where cattle were exposed to serogroup Grippotyphosa exclusively (GRI); farms where cattle were exposed to various serogroups, including Grippotyphosa (GRI – others); and farms where cattle were exposed to various serogroups other than Grippotyphosa (others). Data are not shown for 10 uninfected rodents (1 from site A and 9 from site B).

determine the serogroup present because the *Leptospira* DNA was poor in quality and quantity. Despite the caveats mentioned above, our results suggest that, like elsewhere, Norway rats could potentially be reservoirs of L. *interrogans* [45,46] and thus could have been responsible for the human case of leptospirosis in 2018.

Nutrias. Like Norway rats, nutrias seem to be able to carry *Leptospira* without experiencing significant health consequences [47,48], which is consistent with the absence of any organ lesions in this study. *Leptospira* prevalence was lower in nutrias than in Norway rats, a finding that is consistent with previous epidemiological surveys of nutrias in France [49,50]. In additional studies, *L. interrogans* was detected in nutrias but could not be genotyped to determine serogroup identity [51,52]. Further efforts should be made to characterize the *Leptospira* present and, eventually, confirm whether the serogroups Australis and Icterohaemorrhagiae predominate, as suggested by past MAT results [50]. Compared to Norway rats, nutrias likely contribute less to the maintenance of L. *interrogans*. That said, in this ecosystem, nutrias density can increase drastically, and the relative abundance of a host species is an important consideration when estimating the risk of leptospirosis faced by humans.

Muskrats. The prevalence values observed here and elsewhere [49,50] suggest that muskrats could play a more important role than nutrias as *Leptospira* carriers. However, muskrats were scarce among our field specimens and have a low level of occurrence in the focal ecosystem, which means that they likely make a limited contribution to environmental contamination by *Leptospira*. We found that two of the three infected muskrats were carrying L. *interrogans*. This finding is inconsistent with a previous serological study suggesting that muskrats might be predominantly infected by *Leptospira* serogroup Grippotyphosa, which is frequently associated with L. *krischneri* [50,53]. To our knowledge, this is the first study to describe *Leptospira* species in muskrats. Thus, additional research should be performed to flesh out these preliminary findings and further clarify *Leptospira* carriage in muskrats.

4.2. Source of several human cases of leptospirosis remains unidentified

4.2.1. Leptospira circulating in humans and animals

Epidemiological source tracking compares the identity of the *Leptospira* found in target *versus* potential source populations utilizing preferably DNA or serological data. Uncertainty can arise if data on *Leptospira* circulation dynamics are missing. Although the outbreak reported by Guillois et al. (2018) was attributed to L. *kirschneri* serogroup Grippotyphosa, only 5 of the 14 cases were typed, either *via* serology (4 *Leptospira* serogroup Grippotyphosa) or molecular testing (1 *L. kirschneri*). During the study period (2018 and 2019), there were two cases of leptospirosis in humans that were attributed to L. *kirschneri*, suggesting that this species recurrently occurs in the environment. However, it is possible that some of the remaining cases were caused by other *Leptospira* species, such as the case related to L. *interrogans* in 2018. At least two *Leptospira* strains are responsible for human infections, with L. *kirschneri* being the most frequently detected.

Surprisingly, *L. kirschneri* was not detected in the animal samples although it has been responsible for many human infections in the focal ecosystem. Recent research looking at *Leptospira* infection has suggested that placing the rodents' whole bodies in cold storage prior to tissue sampling could lead to alterations in DNA structural integrity and, thus, to underestimates of prevalence [12]. As we employed this cold-storage method, it is likely that we underestimated the true number of infected rodents. Furthermore, we were only able to identify *Leptospira* species for 11 of the 17 PCR-positive rodents. Our cold-storage method could have been responsible for the poor quality of the DNA and the inability to perform genotyping, despite the sensitivity of cPCR testing [35]. This issue could have limited the likelihood of detecting L. *kirschneri*, if present. Like for human cases, it is essential to comprehensively describe the identity and distribution patterns of host-maintained *Leptospira* to reliably characterize the reservoirs behind leptospirosis outbreaks. Crucial to this goal are the methodologies used to sample animal specimens and tissues.

4.2.2. Animal sampling

The mammal source of the human cases caused by L. *kirschneri* could be a species yet to be sampled. Although we collected the main reported carriers of *Leptospira* in the focal ecosystem, it could be that another animal species was responsible for the above cases. In Western Europe, *L. kirschneri* has also been detected in other small mammals known to occur at sites A and B, such as the wood mouse (*Apodemus sylvaticus*), bank vole (*Myodes glareolus*), greater white-toothed shrew (*Crocidura russula*), crowned shrew (*Sorex coronatus*), and common vole (*Microtus arvalis*) [11,13,54]. Furthermore, in a previous study, a single wood mouse at site A was found to have antibodies against *Leptospira* serogroup Grippotyphosa [8], and the common vole is thought to have caused a leptospirosis outbreak in humans [55]. Taken together, all these findings indicate that L. *kirschneri* is likely circulating among small mammals. Considering that L. *krischneri* serogroup Grippotyphosa can survive 72 h at best under the study conditions [56], it seems probable that *Leptospira* persists in local water resources because hosts are continually shedding bacteria. It is important to investigate the ecology of the small mammals mentioned above and whether or not they could contaminate water resources directly or indirectly, *via* bridge hosts [57]. However, access to samples from wild species is limited, and sampling is extremely restricted in the case of many taxa, such as endangered species, given the need to preserve animal populations, health, and welfare. Some animals, such as mustelids (*e.g.,* species in the genera *Martes* and *Mustela*) could serve as sentinels and be used to characterize the *Leptospira* genotypes circulating among their prey, which are mainly small rodents or other small mammals [58].

4.3. Putting the One Health concept into practice

4.3.1. From a collaborative study to a One Health workforce

In our study, we adopted the One Health approach previously described by the One Health High Level Expert Panel [59] because the recurrence of leptospirosis in kayakers using the Vilaine River is a complex health issue involving a strong interdependence among human, animal, and environmental health. Humans are not the only ones facing health risks because pathogenic *Leptospira* in the environment could cause infections in other susceptible mammals, such as livestock, dogs, and even endangered species (*e.g.,* Eurasian beaver [*Castor fiber*], European mink [*Mustela lutreola*]) [60,61]. Furthermore, control efforts are frequently applied to rodent populations to mitigate public health risks but can elicit animal welfare concerns when evidence for their role as disease reservoirs is weak [62].

Leptospira infections can have severe consequences for human and animal health, underscoring the collective need for leptospire-free water resources and soils, an objective that has brought together multiple sectors, disciplines, and communities. Here, the result has been a multisectorial approach that has exploited human and financial resources provided by institutions focused on human health, animal health, and environmental protection. In addition, data sampling, submission, and analysis have been carried out by various stakeholders and communities (*e.g.,* veterinarians, farmers, kayak professionals, professionals in the domain of health and environmental safety from public and private organizations, academics, and laboratories) and required expertise from various disciplines (*e.g.,* epidemiology, microbiology, pathology, wildlife ecology, population management, and public health). In this regard, our multisectorial and multidisciplinary ecoepidemiological research corresponds to Action Tracks 1.2 and 2.1 in the One Health Joint Plan of Action, which focuses on methods for facilitating One Health work and seeks to understand the drivers behind the emergence of zoonotic pathogens, respectively [31].

Lastly, the collaboration has produced a One Health workforce, as

required by tri- and quadripartite guidelines for building and implementing a One Health framework [30,31]. This workforce can be deployed during future collaborative ecoepidemiological surveys.

4.3.2. *From a One Health workforce to a One Health framework*

Identifying relevant stakeholders is a task required by the tri- and quadripartite guidelines for building and implementing a One Health framework [30,31]. This work was a focal point in our study. More specifically, we have identified the stakeholders that could serve as potential contributors and the actions that they could undertake (Supplemental Data 3). This list will help pinpoint who should be invited to participate in future ecoepidemiological surveys and the specific role they could play. Compared to national or regional stakeholders, local stakeholders may make highly diverse, informal contributions. For example, farmers could report the presence of rodents in their pastures, and everyday citizens could report on any ecosystem changes observed during their recreational activities. Thus, identifying stakeholders is a crucial step that should be taken with care and caution. The support of social scientists can also be sought, which we did not do here.

It is important to fine-tune this One Health framework so that it can better support leptospirosis management and outbreak preparedness. Additionally, the framework must include One Health coordination modalities, action type and timing, task allocation, and instruments for monitoring framework progress. In France, leptospirosis in humans recently became a notifiable disease (Article D-31138 of the Public Health Code modified by the "Décret" n°2023–716). Thus, this One Health framework will be developed against a backdrop of administrative shifts that will facilitate outbreak reporting in the near future.

5. Conclusion

The repeated occurrences of leptospirosis in kayakers sharing the same ecosystem were due to L. *interrogans* and L. *kirschneri* serogroup Grippotyphosa. Our investigation to track down the animal sources of human infections identified the local rodents, nutrias, muskrats and Norway rats as carriers and potential spreaders of *Leptospira interrogans*. However, *L. kirschneri* was not detected in these animal populations. In addition, cattle were not a significant source of *Leptospira* but rather appeared to have been incidentally infected *via* exposure to *Leptospira* in the environment, as is the case for humans. The spreaders of L. *kirschneri* could not be identified in our study and may take part of wildelife other that invasive species. The limited access to wildlife other than invasive species, high-quality samples that can be used for diagnostic testing and DNA sequencing, as well as the gaps in knowledge about the maintenance community were the main challenges to track down the animal source of *Leptospira* outbreaks. Future ecoepidemiological research must be collaborative and multisectorial if we are to progress in describing reservoir communities and the associated risk of leptospirosis within ecosystems.

Author contributions

Conceptualization: A.D., E.L.D., F.A., P.B., G.K., Y.G.; Data curation: A.A., G.L., F.A., D.C., C.L.; Formal analysis: A.C., E.H., K.G., P.B.; Funding acquisition: F.A., M-A.P., Y.G.; Investigation: C.L., G.K.; Methodology: F.A.; Project administration: F.A., M-A.P.; Resources: A.K.; Software: E.H, C.B.; Supervision: G.K., E.L.D; A.K., P.B., F.A.; Validation: F.A.; Visualization: C.B., E.H., F.A.; Roles/Writing - original draft: E.H., F.A.; and Writing - review & editing: A.A., F.A., G.L., M.P., P.B., Y.G.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100726.

References

[1] A.R. Bharti, J.E. Nally, J.N. Ricaldi, M.A. Matthias, M.M. Diaz, M.A. Lovett, P. N. Levett, R.H. Gilman, M.R. Willig, E. Gotuzzo, J.M. Vinetz, Leptospirosis: a zoonotic disease of global importance, Lancet Infect. Dis. 3 (2003) 757–771, https://doi.org/10.1016/S1473-3099(03)00830-2.

[2] P.N. Levett, Systematics of Leptospiraceae, in: B. Adler (Ed.), Leptospira and Leptospirosis, Current Topics in Microbiology and Immunology, Springer, Berlin, Heidelberg, 2015, pp. 11–20.

[3] A.T. Vincent, O. Schiettekatte, C. Goarant, V.K. Neela, E. Bernet, R. Thibeaux, N. Ismail, M.K.N.M. Khalid, F. Amran, T. Masuzawa, R. Nakao, A.A. Korba, P. Bourhy, F.J. Veyrier, M. Picardeau, Revisiting the taxonomy and evolution of pathogenicity of the genus Leptospira through the prism of genomics, PLoS Negl. Trop. Dis. 13 (2019) e0007270, https://doi.org/10.1371/journal.pntd.0007270.

[4] L.G.V. Fernandes, N.E. Stone, C.C. Roe, M.G.A. Goris, H. van der Linden, J.W. Sahl, D.M. Wagner, J.E. Nally, Leptospira sanjuanensis sp. nov., a pathogenic species of the genus Leptospira isolated from soil in Puerto Rico, Int. J. Syst. Evol. Microbiol. 72 (2022) 005560, https://doi.org/10.1099/ijsem.0.005560.

[5] C.G. Nieves, S. Huete, F. Veyrier, M. Picardeau, in: E. Egor Shitikov, I. Moksrousov (Eds.), Taxonomy and Phylogenomics of Leptospira, Academic Press, Cambridge, Massachussets, 2023.

[6] F. Costa, J.E. Hagan, J. Calcagno, M. Kane, P. Torgerson, M.S. Martinez-Silveira, C. Stein, B. Abela-Ridder, A.I. Ko, Global morbidity and mortality of leptospirosis: a systematic review, PLoS Negl. Trop. Dis. 9 (2015) e0003898, https://doi.org/10.1371/journal.pntd.0003898.

[7] B. Fiecek, T. Chmielewski, M. Sadkowska-Todys, M. Czerwiński, G. Zalewska, U. Roguska, S. Tylewska-Wierzbanowska, An outbreak of leptospirosis imported from Germany to Poland, Adv. Clin. Exp. Med. 26 (2017) 415–419, https://doi.org/10.17219/acem/62022.

[8] Y. Guillois, P. Bourhy, F. Ayral, M. Pivette, A. Decors, J.H.A. Grau, B. Champenois, C. Malhère, B. Combes, C. Richomme, M.L. Guyader, L.A. King, A. Septfons, An outbreak of leptospirosis among kayakers in Brittany, North-West France, 2016, Eurosurveillance 23 (2018) 1700848, https://doi.org/10.2807/1560-7917.ES.2018.23.48.1700848.

[9] M. Picardeau, Virulence of the zoonotic agent of leptospirosis: still terra incognita? Nat. Rev. Microbiol. 15 (2017) 297–307, https://doi.org/10.1038/nrmicro.2017.5.

[10] F. Ayral, Z. Djelouadji, V. Raton, A.-L. Zilber, P. Gasqui, E. Faure, F. Baurier, G. Vourc'h, A. Kodjo, B. Combes, Hedgehogs and mustelid species: major carriers of pathogenic Leptospira, a survey in 28 animal species in France (20122015), PLoS One 11 (2016), https://doi.org/10.1371/journal.pone.0162549.

[11] S. Fischer, A. Mayer-Scholl, C. Imholt, N.G. Spierling, E. Heuser, S. Schmidt, D. Reil, U.M. Rosenfeld, J. Jacob, K. Nöckler, R.G. Ulrich, Leptospira genomospecies and sequence type prevalence in small mammal populations in Germany, Vector Borne Zoonotic Dis. 18 (2018) 188–199, https://doi.org/10.1089/vbz.2017.2140.

[12] E. Harran, A. Pinot, A. Kodjo, Z. Djelouadji, M. Le Guyader, S. Sionfoungo Daouda, K. Groud, V. Lattard, F. Ayral, Identification of Pathogenic *Leptospira kirschneri* Serogroup Grippotyphosa in Water Voles (*Arvicola terrestris*) from Ruminant Pastures in Puy-de-Dôme, Central France, Pathogens 12 (2023) 260, https://doi.org/10.3390/pathogens12020260.

[13] A. Obiegala, D. Woll, C. Karnath, C. Silaghi, S. Schex, S. Eßbauer, M. Pfeffer, Prevalence and genotype allocation of pathogenic leptospira species in small mammals from various habitat types in Germany, PLoS Negl. Trop. Dis. 10 (2016) e0004501, https://doi.org/10.1371/journal.pntd.0004501.

[14] C. Munoz-Zanzi, E. Groene, B.M. Morawski, K. Bonner, F. Costa, E. Bertherat, M. C. Schneider, A systematic literature review of leptospirosis outbreaks worldwide, 1970–2012, Rev. Panam. Salud Publica 44 (2020) 1, https://doi.org/10.26633/RPSP.2020.78.

[15] S. Brockmann, I. Piechotowski, O. Bock-Hensley, C. Winter, R. Oehme, S. Zimmermann, K. Hartelt, E. Luge, K. Nöckler, T. Schneider, K. Stark, A. Jansen, Outbreak of leptospirosis among triathlon participants in Germany, 2006, BMC Infect. Dis. 10 (2010) 91, https://doi.org/10.1186/1471-2334-10-91.
[16] A. Perra, V. Servas, G. Terrier, D. Postic, G. Baranton, G. André-Fontaine, V. Vaillant, I. Capek, Cas groupés de Leptospirose à Rochefort, juin 2001, Eurosurveillance 7 (2002) 131–136, https://doi.org/10.2807/esm.07.10.00361-fr.
[17] M.G. Roberts, J.A.P. Heesterbeek, Characterizing reservoirs of infection and the maintenance of pathogens in ecosystems, J. R. Soc. Interface 17 (2020) 20190540.
[18] K.R. Blasdell, S. Morand, S.G.W. Laurance, S.L. Doggett, A. Hahs, D. Perera, C. Firth, Rats in the city: implications for zoonotic disease risk in an urbanizing world, bioRxiv, 2021, https://doi.org/10.1101/2021.03.18.436089, 2021.03.18.436089.
[19] J.M. Vinetz, G.E. Glass, C.E. Flexner, P. Mueller, D.C. Kaslow, Sporadic urban leptospirosis, Ann. Intern. Med. 125 (1996) 794–798, https://doi.org/10.7326/0003-4819-125-10-199611150-00002.
[20] W.A. Ellis, Animal leptospirosis, Curr. Top. Microbiol. Immunol. 387 (2015) 99–137, https://doi.org/10.1007/978-3-662-45059-8_6.
[21] M. Viana, R. Mancy, R. Biek, S. Cleaveland, P.C. Cross, J.O. Lloyd-Smith, D. T. Haydon, Assembling evidence for identifying reservoirs of infection, Trends Ecol. Evol. 29 (2014) 270–279.
[22] X. Wan, C. Yan, Z. Wang, Z. Zhang, Sustained population decline of rodents is linked to accelerated climate warming and human disturbance, BMC Ecol. Evol. 22 (2022) 102, https://doi.org/10.1186/s12862-022-02056-z.
[23] G. Andre-Fontaine, F. Aviat, C. Thorin, Waterborne leptospirosis: survival and preservation of the virulence of pathogenic Leptospira spp, Fresh Water, Curr Microbiol 71 (2015) 136–142, https://doi.org/10.1007/s00284-015-0836-4.
[24] E.V. Karaseva, Yu.G. Chernukha, T.F. Sakhartseva, Results of the investigation of soil for contamination with pathogenic leptospires, Folia Parasitol. 24 (1977) 301–304.
[25] F. Velardo, H. Bouziri, L. Adélaïde, E. Oliosi, M. Layan, A. Descamps, D. Berthod, A. R. Patlán-Hernández, M. Ledrans, M. Pivette, M. Lefort, J. Roux, P. Crépey, A cross-sectional study on infectious health risks regarding freshwater sports practice in Brittany, France, J. Water Health 20 (2022) 356–368, https://doi.org/10.2166/wh.2022.232.
[26] M.C. Schneider, M. Jancloes, D.F. Buss, S. Aldighieri, E. Bertherat, P. Najera, D. I. Galan, K. Durski, M.A. Espinal, Leptospirosis: a silent epidemic disease, Int. J. Environ. Res. Public Health 10 (2013) 7229–7234, https://doi.org/10.3390/ijerph10127229.
[27] M.M. Pereira, M.C. Schneider, C. Munoz-Zanzi, F. Costa, J. Benschop, R. Hartskeerl, J. Martinez, M. Jancloes, E. Bertherat, A road map for leptospirosis research and health policies based on country needs in Latin America, Rev. Panam. Salud Publica 41 (2018) e131, https://doi.org/10.26633/RPSP.2017.131.
[28] Anonymous, Leptospirosis occurences in kayakers between 2018 and 2019, Personnal communication with the French National Public Health Agency, 2023.
[29] P.N. Levett, Leptospirosis, Clin. Microbiol. Rev. 14 (2001) 296–326, https://doi.org/10.1128/CMR.14.2.296-326.2001.
[30] Anonymous, Taking a Multisectoral, One Health Approach: A Tripartite Guide to Addressing Zoonotic Diseases in Countries, Food and Agriculture Organization of the United Nations (FAO), World Organisation for Animal Health (founded OIE) and World Health Organization (WHO), 2019.
[31] Anonymous, One Health Joint Plan of Action, 2022–2026, Food and Agriculture Organization of the United Nations (FAO), United Nations Environment Programme (UNEP), World Health Organization (WHO), World Organisation for Animal Health (founded as OIE), 2022, https://doi.org/10.4060/cc2289en.
[32] A. Chakraborty, S. Miyahara, S.Y. Villanueva, M. Saito, N.G. Gloriani, S. Yoshida, A novel combination of selective agents for isolation of Leptospira species, Microbiol. Immunol. 55 (2011) 494–501.
[33] M. Picardeau, Diagnosis and epidemiology of leptospirosis, Med. Mal. Infect. 43 (2013) 1–9, https://doi.org/10.1016/j.medmal.2012.11.005.
[34] J.F. Toussaint, C. Sailleau, E. Breard, S. Zientara, K. De Clercq, Bluetongue virus detection by two real-time RT-qPCRs targeting two different genomic segments, J. Virol. Methods 140 (2007) 115–123, https://doi.org/10.1016/j.jviromet.2006.11.007.
[35] F. Merien, P. Amouriaux, P. Perolat, G. Baranton, I. Saint Girons, Polymerase chain reaction for detection of Leptospira spp. in clinical samples, J. Clin. Microbiol. 30 (1992) 2219–2224.
[36] F. Merien, D. Portnoi, P. Bourhy, F. Charavay, A. Berlioz-Arthaud, G. Baranton, A rapid and quantitative method for the detection of Leptospira species in human leptospirosis, FEMS Microbiol. Lett. 249 (2005) 139–147.
[37] World Organisation for Animal Health (founded as OIE), Leptospirosis, in: OIE Terrestrial Manual 2021, 8e Ed, 2018, p. 1833.
[38] M.D. Miller, K.M. Annis, M.R. Lappin, K.F. Lunn, Variability in results of the microscopic agglutination test in dogs with clinical leptospirosis and dogs vaccinated against leptospirosis, J. Vet. Intern. Med. 25 (2011) 426–432, https://doi.org/10.1111/j.1939-1676.2011.0704.x.
[39] F.C. Ayral, D.J. Bicout, H. Pereira, M. Artois, A. Kodjo, Distribution of leptospira serogroups in cattle herds and dogs in France, Am. J. Trop. Med. Hyg. 91 (2014) 756–759, https://doi.org/10.4269/ajtmh.13-0416.
[40] J. Naudet, L. Crespin, J. Cappelle, A. Kodjo, F. Ayral, Circulating serogroups of Leptospira in swine from a 7-year study in France (2011-2017), Porcine Health Manag 8 (2022) 15, https://doi.org/10.1186/s40813-022-00257-y.
[41] J. Wagenaar, R.L. Zuerner, D. Alt, C.A. Bolin, Comparison of polymerase chain reaction assays with bacteriologic culture, immunofluorescence, and nucleic acid hybridization for detection of Leptospira borgpetersenii serovar hardjo in urine of cattle, Am. J. Vet. Res. 61 (2000) 316–320, https://doi.org/10.2460/ajvr.2000.61.316.
[42] F. Fornazari, R. Costa da Silva, V.B. Richini-Pereira, H.E.O. Beserra, M.C. R. Luvizotto, H. Langoni, Comparison of conventional PCR, quantitative PCR, bacteriological culture and the Warthin starry technique to detect Leptospira spp. in kidney and liver samples from naturally infected sheep from Brazil, J. Microbiol. Methods 90 (2012) 321–326, https://doi.org/10.1016/j.mimet.2012.06.005.
[43] F. Ayral, J. Artois, A.-L. Zilber, F. Widen, K.C. Pounder, D. Aubert, D.J. Bicout, M. Artois, The relationship between socioeconomic indices and potentially zoonotic pathogens carried by wild Norway rats: a survey in Rhône, France (2010−2012), Epidemiol. Infect. 143 (2015) 586–599, https://doi.org/10.1017/S0950268814001137.
[44] C.G. Himsworth, J. Bidulka, K.L. Parsons, A.Y.T. Feng, P. Tang, C.M. Jardine, T. Kerr, S. Mak, J. Robinson, D.M. Patrick, Ecology of Leptospira interrogans in Norway Rats (Rattus norvegicus) in an Inner-City neighborhood of Vancouver, Canada, PLoS Negl. Trop. Dis. 7 (2013) e2270, https://doi.org/10.1371/journal.pntd.0002270.
[45] A.B. Thiermann, The Norway rat as a selective chronic carrier of Leptospira icterohaemorrhagiae, J. Wildl. Dis. 17 (1981) 39–43, https://doi.org/10.7589/0090-3558-17.1.39.
[46] F. Ayral, A.-L. Zilber, D.J. Bicout, A. Kodjo, M. Artois, Z. Djelouadji, Distribution of Leptospira interrogans by multispacer sequence typing in urban Norway rats (Rattus norvegicus): a survey in France in 2011-2013, PLoS One 10 (2015) e0139604, https://doi.org/10.1371/journal.pone.0139604.
[47] S.A. Waitkins, S. Wanyangu, M. Palmer, The coypu as a rodent reservoir of leptospira infection in Great Britain, J Hyg (Lond) 95 (1985) 409–417.
[48] D.A. Athanazio, E.F. Silva, C.S. Santos, G.M. Rocha, M.A. Vannier-Santos, A.J. A. McBride, A.I. Ko, M.G. Reis, Rattus norvegicus as a model for persistent renal colonization by pathogenic Leptospira interrogans, Acta Trop. 105 (2008) 176–180, https://doi.org/10.1016/j.actatropica.2007.10.012.
[49] F. Aviat, B. Blanchard, V. Michel, B. Blanchet, C. Branger, J. Hars, F. Mansotte, L. Brasme, C. De Champs, P. Bolut, P. Mondot, J. Faliu, S. Rochereau, A. Kodjo, G. Andre-Fontaine, Leptospira exposure in the human environment in France: a survey in feral rodents and in fresh water, Comp. Immunol. Microbiol. Infect. Dis. 32 (2009) 463–476, https://doi.org/10.1016/j.cimid.2008.05.004.
[50] F. Ayral, A. Kodjo, G. Guédon, F. Boué, C. Richomme, Muskrats are greater carriers of pathogenic Leptospira than coypus in ecosystems with temperate climates, PLoS One 15 (2020) e0228577, https://doi.org/10.1371/journal.pone.0228577.
[51] E. Mazzotta, L. Bellinati, C. Bertasio, M.B. Boniotti, L. Lucchese, L. Ceglie, F. Martignago, S. Leopardi, A. Natale, Synanthropic and wild animals as sentinels of zoonotic agents: a study of Leptospira genotypes circulating in northeastern Italy, Int. J. Environ. Res. Public Health 20 (2023) 3783, https://doi.org/10.3390/ijerph20053783.
[52] J. Vein, Evaluation of the Role of the Coypu (*Myocastor coypus*) in the Epidemiological Cycle of Leptospirosis and in the Environmental Contamination in Wetlands : A Study in Two Populations in the East of France, 2013.
[53] L. Salaün, F. Mérien, S. Gurianova, G. Baranton, M. Picardeau, Application of multilocus variable-number tandem-repeat analysis for molecular typing of the agent of leptospirosis, J. Clin. Microbiol. 44 (2006) 3954–3962, https://doi.org/10.1128/JCM.00336-06.
[54] A. Mayer-Scholl, J.A. Hammerl, S. Schmidt, R.G. Ulrich, M. Pfeffer, D. Woll, H. C. Scholz, A. Thomas, K. Nöckler, Leptospira spp. in Rodents and Shrews in Germany, Int. J. Environ. Res. Public Health 11 (2014) 7562–7574, https://doi.org/10.3390/ijerph110807562.
[55] S. Desai, U. van Treeck, M. Lierz, W. Espelage, L. Zota, A. Sarbu, M. Czerwinski, M. Sadkowska-Todys, M. Avdicová, J. Reetz, E. Luge, B. Guerra, K. Nöckler, A. Jansen, Resurgence of field fever in a temperate country: an epidemic of leptospirosis among seasonal strawberry harvesters in Germany in 2007, Clin. Infect. Dis. 48 (2009) 691–697, https://doi.org/10.1086/597036.
[56] L.H. Nau, D. Emirhar, A. Obiegala, M. Mylius, M. Runge, J. Jacob, N. Bier, K. Nöckler, C. Imholt, D. Below, C. Princk, J. Dreesman, R.G. Ulrich, M. Pfeffer, A. Mayer-Scholl, Leptospirose in Deutschland: Aktuelle Erkenntnisse zu Erregerspezies, Reservoirwirten und Erkrankungen bei Mensch und Tier, Bundesgesundheitsbl 62 (2019) 1510–1521, https://doi.org/10.1007/s00103-019-03051-4.
[57] A. Caron, J. Cappelle, G.S. Cumming, M. de Garine-Wichatitsky, N. Gaidet, Bridge hosts, a missing link for disease ecology in multi-host systems, Vet. Res. 46 (2015) 83, https://doi.org/10.1186/s13567-015-0217-9.
[58] F. Ayral, Z. Djelouadji, V. Raton, A.-L. Zilber, P. Gasqui, E. Faure, F. Baurier, G. Vourc'h, A. Kodjo, B. Combes, Hedgehogs and mustelid species: major carriers of pathogenic Leptospira, a survey in 28 animal species in France (20122015), PLoS One 11 (2016) e0162549, https://doi.org/10.1371/journal.pone.0162549.
[59] O.H.H.L.E. Panel (OHHLEP), W.B. Adisasmito, S. Almuhairi, C.B. Behravesh, P. Bilivogui, S.A. Bukachi, N. Casas, N.C. Becerra, D.F. Charron, A. Chaudhary, J.R. C. Zanella, A.A. Cunningham, O. Dar, N. Debnath, B. Dungu, E. Farag, G.F. Gao, D. T.S. Hayman, M. Khaitsa, M.P.G. Koopmans, C. Machalaba, J.S. Mackenzie, W. Markotter, T.C. Mettenleiter, S. Morand, V. Smolenskiy, L. Zhou, One health: a new definition for a sustainable and healthy future, PLoS Pathog. 18 (2022) e1010537, https://doi.org/10.1371/journal.ppat.1010537.
[60] M. Moinet, C. Fournier-Chambrillon, G. André-Fontaine, S. Aulagnier, A. Mesplède, B. Blanchard, V. Descarsin, P. Dumas, Y. Dumas, C. Coïc, L. Couzi, P. Fournier, Leptospirosis in free-ranging endangered European mink (Mustela lutreola) and other small carnivores (Mustelidae, Viverridae) from southwestern France,

J. Wildl. Dis. 46 (2010) 1141–1151, https://doi.org/10.7589/0090-3558-46.4.1141.

[61] N. Marreros, S. Zürcher-Giovannini, F.C. Origgi, Z. Djelouadji, J. Wimmershoff, M. Pewsner, E. Akdesir, M. Batista Linhares, A. Kodjo, M.-P. Ryser-Degiorgis, Fatal leptospirosis in free-ranging Eurasian beavers (Castor fiber L.), Switzerland, Transbound. Emerg. Dis. 65 (2018) 1297–1306, https://doi.org/10.1111/tbed.12879.

[62] A. Mankad, U. Kennedy, L. Carter, Biological control of pests and a social model of animal welfare, J. Environ. Manag. 247 (2019) 313–322, https://doi.org/10.1016/j.jenvman.2019.06.080.