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Original article

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ABSTRACT

Objective: A large and unprecedented outbreak of an attenuated form of hemorrhagic fever with renal syndrome called nephropathia epidemica (NE) and caused by Puumala virus (PUUV) occurred in 2021 in the southern Jura Mountains (France) leading to numerous hospitalizations. The aim of this study was to investigate the circulation of PUUV in its animal reservoir at the time of this outbreak.
Methods: We conjointly surveyed bank vole relative abundance, small mammal community composition, and PUUV circulation in bank voles (seroprevalence and genetic diversity) in the Jura NE epidemic area, between 2020 and 2022.
Results: Trapping results showed a higher relative abundance of bank voles in 2021 compared to 2020 and 2022. Extremely high levels of PUUV seroprevalence in bank voles were found at the time of the human NE epidemic with seropositive animals trapped in almost all trap lines as of spring 2021. Genetic analyses of PUUV (S segment) gathered in 2021 at two sampling sites revealed a strong clustering of these strains within the "Jura" clade. No significant genetic variation was detected compared to what was already known to be circulating in the Jura region.
Conclusion: These results underline a need for enhanced monitoring of PUUV circulation in host reservoir populations in NE endemic areas. This would enable the relevant actors to better inform and sensitize the public on this zoonotic risk, and to implement prevention strategies in collaboration with physicians.

1. Introduction

Over the last two decades, human diseases associated with orthohantaviruses have been recognized as a growing public health concern worldwide. These zoonotic rodent-borne RNA viruses are transmitted to humans by biting or via inhalation of aerosolised virus in contaminated rodent urine and feces. They can cause hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary (or cardiopulmonary) syndrome (HPS). In France, the main agent of orthohantavirus infections is Puumala virus (PUUV). It induces an attenuated form of HFRS, nephropathia epidemica (NE), with an average of 100 human hospitalized cases (lethality ≈ 0.4%) detected annually [1]. Like other orthohantaviruses, PUUV has a unique and specific natural reservoir host, the bank vole *Myodes glareolus*, which is a forest-dwelling rodent species [2] and develops chronic infection without (at least minimally visible) clinical signs [3]. While *M. glareolus* exhibits spatially continuous distribution all over France, except on the Mediterranean coast, human cases are mostly reported in the northeastern part of the country. Krug et al. [4] confirmed that seroprevalence among forestry workers, who are highly exposed to PUUV risk, was high in these known endemic areas, particularly in the Franche-Comté region.

In 2021, a large-scale outbreak of NE was detected in the southern Jura Mountains of France [5]. It led to numerous hospitalizations, dialysis treatment and one death. If this region has long been known as an endemic area for NE, the 2021 epidemic was unprecedented in its amplitude [6]. As early as March, the number of cases detected was abnormally high (Jura administrative department). The number of human cases detected in 2021 reached 205 for this geographic area, which was by far the highest level recorded since surveillance began in France (data CNR Hantavirus,

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available at https://www.pasteur.fr/fr/sante-publique/CNR/les-cnr/hantavirus/rapports-d-activite). As of now, the factors leading to epidemic peaks are still not well-understood [6,7].

In Europe, PUUV transmission between humans has never been reported. The risk factors for humans are related to human behaviours and forest activities liable to increase exposure to PUUV [8]. Evidence suggests that human NE epidemics in temperate areas are correlated with the abundance of bank voles, but this is not necessarily the case in all regions [9]. Higher numbers of human cases are detected during the peak [10,11] or increasing [12] phase of multi-annual bank vole population fluctuations, when seroprevalence and incidence of PUUV in bank voles are at their highest levels, suggesting that a PUUV epizootic in bank voles could lead to NE outbreak in humans [11,13]. Variations of bank vole abundance are driven by several environmental factors, including landscape connectivity, vegetation features, food availability, climatic conditions and soil composition [13–15]. These fluctuations directly affect PUUV transmission dynamics and persistence in the bank vole populations. Abiotic characteristics can also indirectly influence PUUV epidemiology. They can affect bank vole behaviour [16] and genetic diversity [17–19], which can impact PUUV transmission through changes in exposure or susceptibility. In addition, these environmental features can shape PUUV epidemiology by influencing the virus's ability to survive outside its reservoir host [20], consequently affecting the potential transmission period [8,21].

Genetically differentiated PUUV variants could also have different dynamics in their hosts or lead to different levels of excretion, which could in turn affect PUUV transmission or virulence [22]. A previous study showed that differentiated clades of PUUV circulated in bank voles from endemic and non-endemic areas of France [23]. The genetic features that differentiate these PUUV variants could underlie differences in their transmission and/or virulence in humans. This could at least partially mediate the different levels of PUUV seroprevalence observed in forest workers laboring in these areas [4], notwithstanding the similar levels of PUUV seroprevalence observed in bank vole populations [24].

Finally, the biodiversity of small mammal communities may influence PUUV transmission within bank vole populations, and consequently NE risk, through diversified mechanisms. They encompass the "dilution effect", *i.e.,* the decrease of disease risk with increasing vertebrate diversity [25,26]. Several empirical studies on PUUV have shown evidence of the dilution effect, as the presence of the common shrews, field voles, or wood mice is associated with decreased densities or encounter rates between bank voles and lower viral transmission [15,27]. Pronounced species diversity may dilute PUUV transmission by decreasing the abundance of bank voles through the effects of interspecific competition, or by reducing the number of aggressive encounters between them. These results advocate for the role of biodiversity conservation in limitation of the zoonotic risks associated with orthohantaviruses.

Uncovering the links between reservoir host and virus dynamics or evolution requires multidisciplinary research [6], of which the results should help to anticipate epidemic peaks and to implement future preventive actions. In this study, we monitored bank vole populations for three consecutive years (between 2020 and 2022) in the region where the 2021 French NE outbreak occurred. Our first objective was to estimate PUUV seroprevalence during this period and to analyse it with regard to (a) bank vole population abundance and (b) the diversity of the small mammal community. Managed and protected forests were surveyed so as to include potentially contrasted levels of small mammal biodiversity. Our second objective was to genetically characterize the PUUV strains circulating in these bank vole populations during this period. We compared them with the PUUV strains having circulated there 15 years prior. We aimed at assessing the genetic evolution of PUUV in this region so as to determine whether the introduction of new variants or significant genetic changes resulting from rapid and recent evolution could be linked to the 2021 NE outbreak.

2. Material and methods

2.1. Sampling

Rodent trapping and data collection are detailed in [28]. Briefly, bank voles were live-trapped five times between June 2020 and June 2022 at two sites in the Jura Mountains, Mignovillard (FRFMIG), a forest managed by the National Forest Office (ONF), and "La Glaciere" (FRFGLA), a forested biological reserve located a few km from Mignovillard (Fig. 1). These sites are 25–70 km distant from the hospitals in which the human cases described in [5] were recorded. Sampling was allowed only in June and October 2021 in FRFGLA for ethical reasons. Six to ten lines of 20 live-traps (INRA model, composed of an aluminum tunnel coupled with a plastic rest box) were set up so that each sampling site consisted of an area of several km^2. Trapping sessions per site lasted at least three nights, except when abundances were too low and new trap lines had to be set up for a few more nights. Animal dissections and measurements were performed according to the protocols described in [29]. Capture data are registered in the CBGP small mammal database (BPM, https://bpm-cbgp.science; associated biological samples (organs, blood, feces) are included in the CBGP reference collection of small mammals (https://doi.org/10.15454/WWNUPO). All animal studies were conducted in accordance with French and European regulations on the care and protection of laboratory animals (French Law 2013-118 from February 1st, 2013 and Directive 2010/63/EU from September 22, 2010). All the animal procedures were performed with the approval of the competent ethics committee (C2EA-LR) and the "Direction Départementale de la Protection des Populations de l'Hérault" (E 34-169-1 Agreement).

Trapping success (T_r) was used as a proxy for relative rodent abundance [30]. It was calculated from the capture results of the first three nights of trapping according to the formula $T_r = \ln(1 - \text{number of rodents trapped}/(\text{number of traps} \times \text{number of nights})) \times (-100)$.

2.2. Serological analyses

Bank vole blood samples were screened for anti-PUUV IgG using immunofluorescence assay (IFA) as defined previously [31]. Briefly, slide wells coated with a mix of PUUV Sotkamo strain infected and non-infected Vero E6 cells at a ratio of 1:1 were challenged with the samples. The wells were covered with blood samples diluted with phosphate-buffered saline (PBS) at a ratio of 1:10. PUUV-positive human serum sample was used as a positive control and PBS was used as a negative control. After the initial incubation, the slides were washed and incubated with a secondary antibody. Fluorescein (FITC) AffiniPure Goat Anti-Mouse IgG (H + L) (Jackson ImmunoResearch Ltd., UK) was used to detect mouse IgG in test samples and negative controls, and Fluorescein (FITC) AffiniPure Goat Anti-Human IgG (H + L) (Jackson ImmunoResearch Ltd., UK) to detect human IgG in positive controls. The samples were assessed and evaluated based on the fluorescence emitted by the secondary antibody. Only the seropositive samples were directed to sequencing.

We performed a GLM with a binomial family and a logit function to analyze the impact of explanatory variables (sex, weight, locality and sampling period) on PUUV seroprevalence. Statistical modeling was performed in R v4.1.3 using the R stats and MuMIn

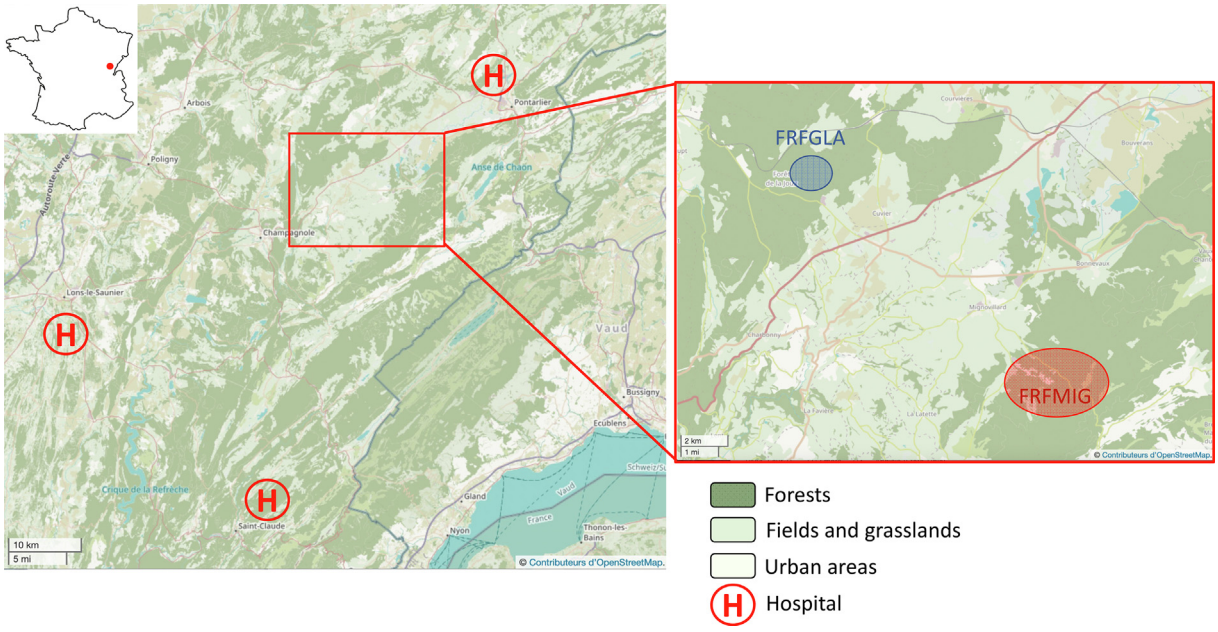

Fig. 1. Map showing (a) the Jura department in France, the three localities (Lons-le-Saunier, Pontarlier, and Saint Claude) where the hospitals having provided the PUUV data used in Brun et al. [5] are located (symbol "H"), and the Mignovillard area that is the focus of this study; (b) the two sites surveyed here, FRFMIG, the managed forest of Mignovillard and FRFGLA, a protected forest in Censeau, La Glaciere. Forest areas are indicated in dark green, fields and grasslands in light green, and urban areas in beige.

packages, and the glm function. The best model was selected based on the AICc criteria.

2.3. Viral RNA extraction and sequencing

Viral RNA was extracted from the lungs of seropositive voles using the QIAamp Viral Mini Kit (Qiagen) and quantified using a Nanodrop 8000 spectrophotometer (Thermo Scientific); 1500 ng of viral RNA were reverse-transcribed to cDNA with SuperScript III First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. This study focused on the S segment of PUUV as there were too few M or L sequences available in GenBank from this French area. This limitation would have prevented further analyses of PUUV genetic evolution over time.

RT-PCRs primers and PCR conditions are indicated in Supporting file S1. PCR products were verified by electrophoresis on 2% agarose gel. The PCR Clean-up Gel Extraction kit (Macherey-Nagel) was used to purify the specific amplicon of PCR2 if needed. PCR products were sequenced using Sanger method in both directions by Eurofins genomics.

The S coding sequences of the 11 samples (9 FRFMIG and 2 FRFGLA) that were successfully or almost completely sequenced were deposited in the GenBank database under accession numbers OQ714391-OQ714401.

2.4. Phylogenetic analyses

We performed phylogenetic analyses on the dataset composed of all the complete S segment sequences from PUUV (open reading frame of 1299 nucleotides encoding the viral nucleocapsid protein) available from GenBank and corresponding to French bank voles, human cases from Jura as well as the new S segment sequences obtained in this study. Sequences of German PUUV strain recovered from a bank vole (Mu/07/1219, GenBank number KJ994776) and of Hokkaido Genotype of Puumala Virus from the grey red-backed vole (Kamiiso_8cr_95; AB010730) were used as outgroups.

Phylogenetic analyses were performed as described in [23]. The optimal substitution model was identified as the general time reversible (GTR) + G model with SMS software.

The estimate of genetic divergence between sequences at the amino acid level was calculated using a function implemented in the Mega v11.0 program. All parameters were set to their default value. All ambiguous positions were removed for each sequence pair.

PUUV evolutionary rate within the surveyed geographic area was estimated using TempEst v1.5.1 software.

3. Results

3.1. Trapping success

Rodent trapping resulted in the capture of 190 bank voles *Myodes glareolus*, 54 wood mice *Apodemus sylvaticus* and 122 yellow-necked mice *Apodemus flavicollis* (Fig. 2). *M. glareolus* was the most abundant species in all sampling sites and periods. Trapping success rates peaked in autumn 2021, whatever the trapping sites and rodent species considered. The outbreak was followed by rodent population collapses in spring 2022. In FRFGLA, the composition of the rodent community (based on the trapping success) did not seem to differ markedly from the one detected in FRFMIG.

3.2. PUUV seroprevalence

All in all, 63/190 bank voles were considered PUUV-seropositive by IFA, with marked variations observed between sampling sites and periods (Table 1). Among them, four voles were considered as juveniles and potentially carrying maternal antibodies rather than genuine PUUV infection. The limited sample size of juveniles hindered our ability to analyze a potential juvenile dilution effect [32]. A general linear "logistic regression" model showed that the presence of anti-PUUV antibodies was significantly explained by weight ($p = 0.02$), sampling site and date ($p < 10^{-10}$) (Table S1).

We found high levels of seroprevalence in 2021 in the Mignovillard forest (FRFMIG: 65.6–85.3%). Lower levels of seroprevalence

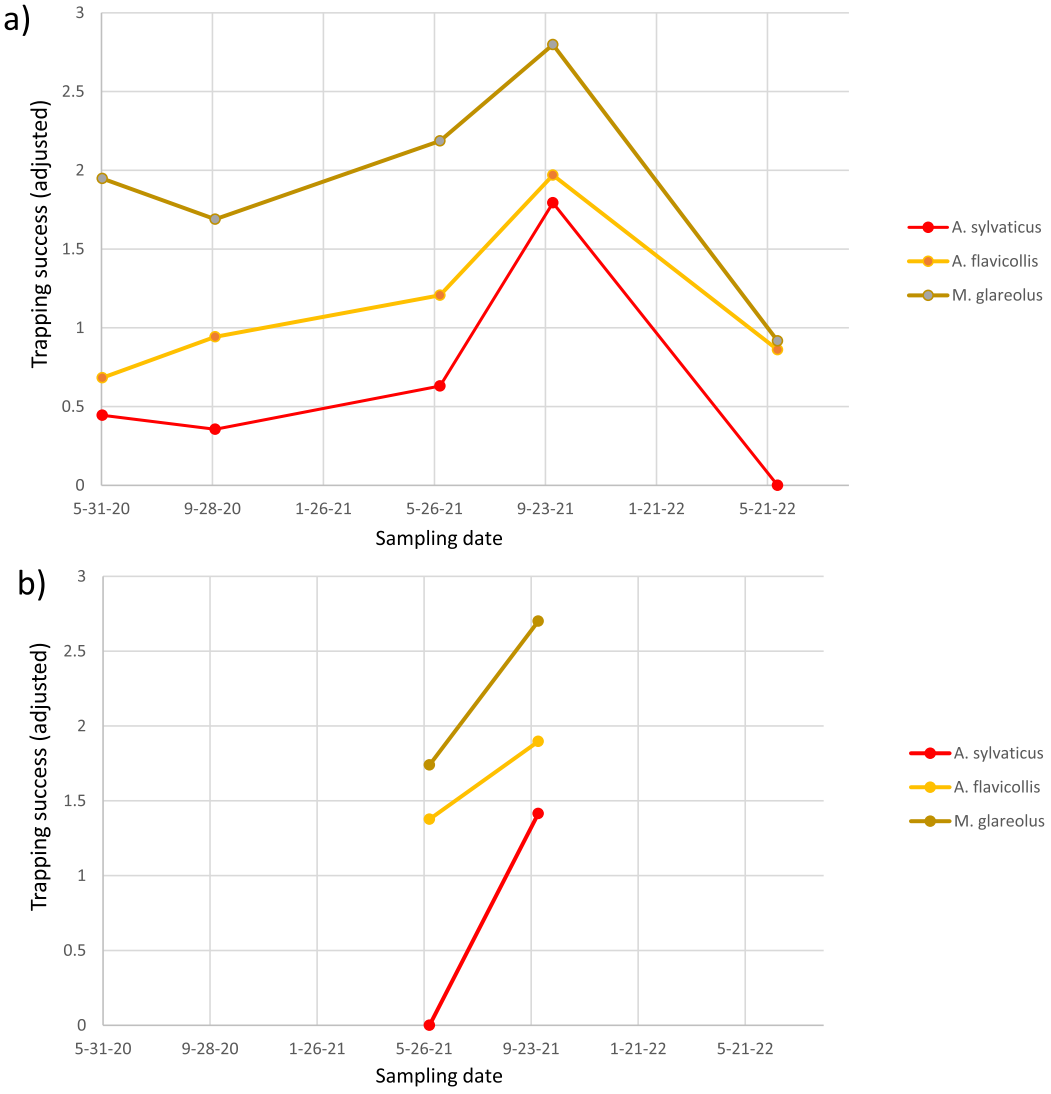

Fig. 2. Rodent trapping success (adjusted, T_r) in (a) FRFMIG and (b) FRFGLA from spring 2020 to spring 2022. Trapping was allowed only at FRFGLA in 2021. Data are presented for the three main species captured, the wood mouse *Apodemus sylvaticus*, the yellow-necked mouse *Apodemus flavicollis*, and the bank vole *Myodes glareolus*.

Table 1
Number of PUUV seropositive bank voles found by Indirect Fluorescent Antibody (IFA) assay over the total number of bank voles analysed, for all sampling sites and dates. The seroprevalence (percentage of seropositive bank voles) is indicated as well as the seroprevalence estimated without considering that were PUUV-seropositive juveniles (potentially carrying maternal antibodies rather than genuine infection. Grey cells correspond to the study site FRFMIG (managed forest of Mignovillard) whereas white cells correspond to the study site FRFGLA (biological reserve of La Glaciere).

	FRFMIG Spring 2020	FRFMIG Autumn 2020	FRFMIG Spring 2021	FRFGLA Spring 2021	FRFMIG Autumn 2021	FRFGLA Autumn 2021	FRFMIG Spring 2022
Number of PUUVs IFA+/ total number of bank voles	0/25	1/35	29/34	2/19	21/32	8/33	2/12
Number of PUUVs IFA + juveniles	0	0	3	0	1	0	0
Seroprevalence	0%	2.8%	85.3%	10.5%	65.6%	24.2%	16.6%
Seroprevalence without PUUV IFA + juveniles	0%	2.8%	76.4%	10.5%	62.5%	24.2%	16.6%

were detected during the same period in the biological reserve La Glaciere (FRFGLA: 10.5–24.2%). Overall levels of seroprevalence were likewise much lower in 2020 and 2022 (between 0% and 16.6%).

While seropositive bank voles were trapped in almost all trap lines in spring and fall 2021 (Fig. 3), they were present only in a highly restricted area (one or two trap lines) in 2020 and 2022.

3.3. PUUV genetic characterization

PUUV phylogenetic analyses showed that the virus strains detected in 2021 clustered together within the "Jura" clade, which corresponds to PUUV sequences obtained from *M. glareolus* captured in localities within the French Jura, between 2005 and 2014 (Fig. 4). The new sequences exhibited amino-acid signatures in the nucleoprotein, which are typical of the "Jura" clade variants in France (e.g. T amino-acid at position 233).

PUUV sequences from FRFMIG and FRFGLA formed three "subclusters". The first included strains circulating in bank voles trapped in 2005–2006, the second included strains from bank voles collected in 2005, 2007 and 2021 in FRFMIG, and the third corresponded to PUUV strains detected from bank voles trapped in 2021 in La Glaciere FRFGLA.

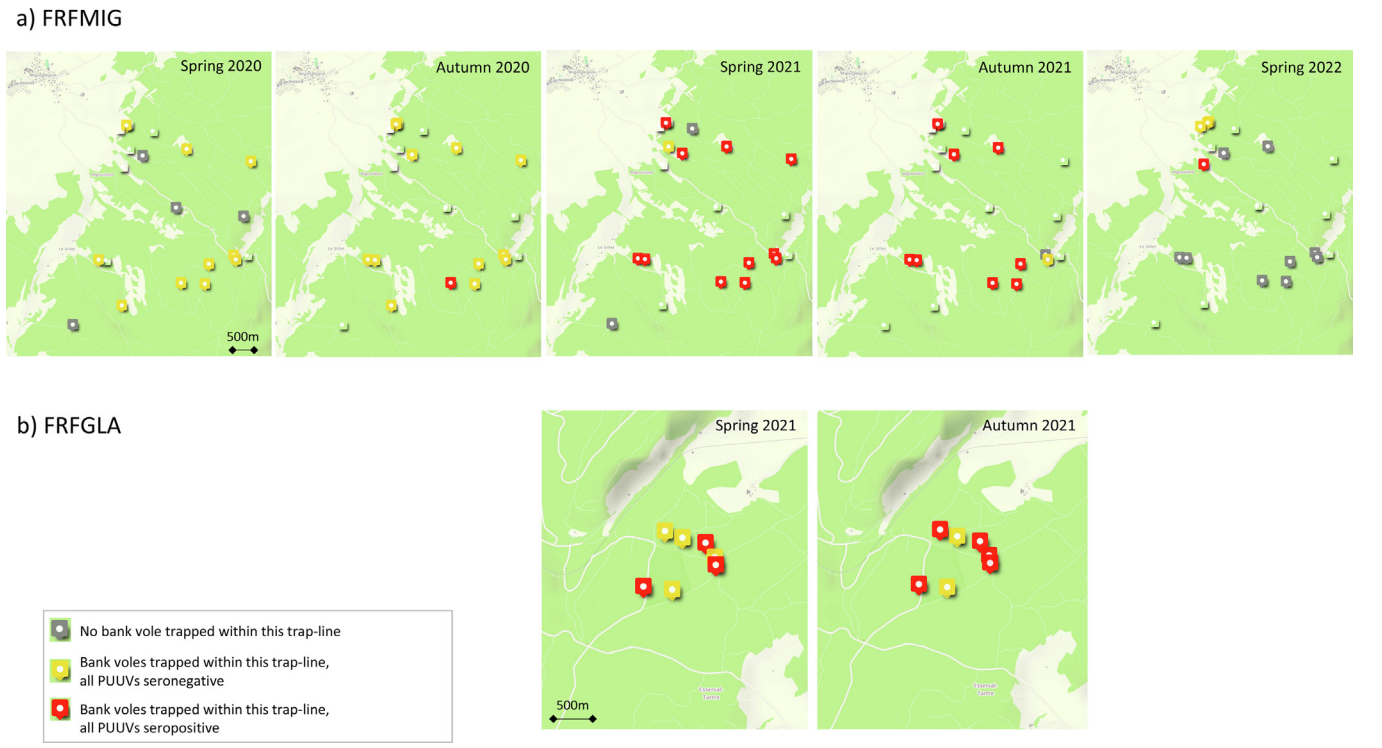

Fig. 3. Qualitative spatiotemporal changes in bank vole trapping and PUUV serology in two sampling sites, (a) the managed forest of Mignovillard (FRFMIG) and (b) the biological reserve of La Glaciere (FRFGLA).

We analyzed the genetic distance between PUUV sequences corresponding to bank voles trapped around Mignovillard since 2005 (Fig. 5). The highest numbers of nucleotide and/or amino-acid differences between sequences were detected between PUUV strains collected at FRFGLA in 2021 and all other sequences from FRFMIG, regardless of sampling year. At the amino-acid level, we detected only a few differences between PUUV sequences found in FRFMIG between 2005 and 2021 (maximum of five amino acid exchanges within 433 amino acid residues of the entire nucleocapsid protein).

The nucleotide substitution rate estimated using root-to-tip regression in TempEst for the whole Mignovillard dataset approximated 1.5×10^{-5} substitutions per site per year.

4. Discussion

We found extremely high levels of PUUV seroprevalence (65.6% and 85.3 %) in bank voles from FRFMIG at the time of the 2021 human NE epidemic in the French Jura [5]. In addition, genetic analyses of PUUV S segment showed that the strains circulating in 2021 clustered with the previously identified "Jura" clade. No significant genetic variation was detected compared to what was already known to have circulated in the Jura region, 15 years previous.

To our knowledge, such high levels of seroprevalence had never been recorded in bank vole populations from France, whereas up to 100% had previously been reported in Germany [9]. In the French Jura, the maximum levels of seroprevalence reached 15.6% [33], 10.0 % [19] and 29.7% [24]. Other studies having investigated PUUV seroprevalence in another endemic area of France, the Ardennes, found maximum levels of about 40% [17,34]. Surprisingly, the seroprevalence levels observed in the bank vole population from FRFGLA, which is only 6 km from FRFMIG, were consistent with these data (10.5% and 24.2%).

Our trapping results show a higher relative abundance of bank voles in 2021 compared to 2020 and 2022, in FRFMIG. Similar patterns were observed for the two *Apodemus* species. This suggested that the peak phase for these small mammals was reached in 2021. Multiannual fluctuations of rodent population abundance are well-known, and they are mainly driven by climatic and resource conditions in temperate forests [35].

Previous studies have demonstrated the close relationship between multiannual abundance cycles and variations in PUUV seroprevalence of bank vole populations [36,37]. Our study corroborated this positive relationship (Fig. S1), with higher numbers of individuals leading to increased PUUV transmission and spread in wild populations in FRFMIG [38]. However, other studies found only limited correlation between PUUV circulation in bank voles and outbreaks in human populations [9]. These contrasting patterns highlight the complex relationship between rodent abundance and prevalence in humans, potentially driven by specific environmental factors influencing the dynamics of bank vole populations or PUUV persistence in the environment [7].

We investigated several hypotheses to explain these extremely high levels of seroprevalence detected in FRFMIG in 2021.

First, by capitalizing on PUUV data previously gathered from bank voles captured in the same geographic area between 2005 and 2007, we tried to determine whether the evolution of PUUV between 2005 and 2021 could have led to new variants. The genetic sequences corresponding to the S segment of the PUUV strains gathered in 2021 at FRFMIG and FRFGLA clustered within the "Jura" clade and displayed the classical signatures of the Jura strains. The persistence of PUUV variants over time has been reported and could be explained by bottleneck events driven by genetic drift or selection processes in the bank vole population [38]. Although our data are not optimal for obtaining a confidence estimate of the evolution rate, molecular clock analysis crudely estimated it as around 10^{-5} substitutions per site per year in the French Jura region. This estimate is lower than those recently cal-

Fig. 4. Maximum Likelihood phylogenetic tree of French PUUV strains (ML, GTR + G nucleotide substitution model) constructed on the basis of complete S segment coding parts. Sequences belonging to the Jura clade are highlighted in grey. PUUV strains collected and sequenced in this study are indicated in red. Previously sequenced PUUV strains from Mignovillard (FRFMIG) are shown in blue. Human sequences are indicated by a red star. Branch support was determined by an aLRT test and is represented by a colour dot at each node. Scale bars indicate numbers of substitutions per nucleotide. PUUV variants from Germany (KJ994776) ^and from Japan (AB010730) were used as outgroups.

culated for the Ardennes area or northwest Germany ($\sim 10^{-4}$ substitutions per site per year, [9,39], suggesting a stable virus with relatively slow evolution at this site during this period. These analyses do not reveal significant genetic changes in PUUV from what was known to circulate in the Jura 15 years ago. The hypotheses of newly introduced strains or of rapid evolution could therefore not explain the high levels of seroprevalence observed. Nevertheless, this conclusion should be taken cautiously as due to a lack of available complete genomes, our analyses were performed only on the nucleoprotein. In the future, it would be interesting to examine whether more important/impacting modifications have occurred in other PUUV proteins.

Second, we analysed the differences between FRFMIG and FRFGLA, and found marked genetic differences between the strains circulating in these two sites in 2021. These spatial differences in geographically close sites were greter than the temporal differences detected between 2005 and 2021 in FRFMIG. This finding aligns with a previous study [9] that demonstrated significant

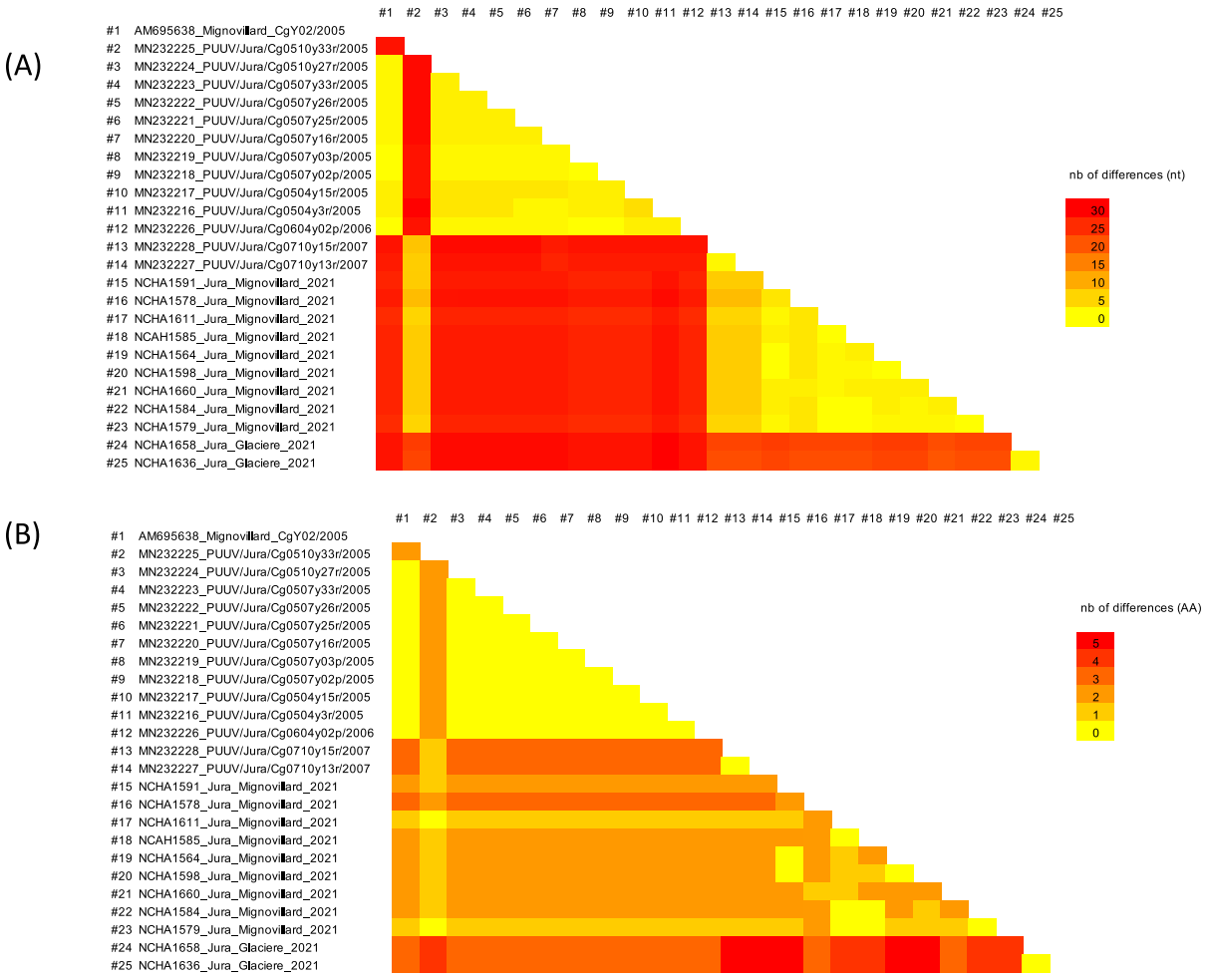

Fig. 5. Heatmap representing the estimates of PUUV evolutionary divergence at the nucleotide (A) and amino-acid (B) levels between sequences gathered from bank voles collected in FRFMIG (Mignovillard) and FRFGLA (La Glaciere) between 2005 and 2021. Colors indicate the number (nb) of differences observed at the nucleotide and amino-acid levels between two PUUV sequences.

genetic differentiation among PUUV strains from geographically close localities. The observed genetic differences should be further explored in the complete genome of PUUV so as to determine their potential impacts on PUUV characteristics, which could influence the epidemiology of NE, including transmission between reservoirs and to humans. In this regard, the availability of complete viral genomes from both human patients and bank voles is critical. By comparing complete genomes of human and rodent PUUV collected from the same geographical areas, we could deepen our understanding of the genetic features associated with enhanced viral transmission from rodents to humans during outbreaks.

Finally, the small mammal communities in FRFMIG and FRFGLA were quite similar, with the same species detected and comparable relative abundance observed for the three main species trapped, namely *M. glareolus*, *A. flavicollis* and *A. sylvaticus*. Moreover, we did not see any trend suggesting that PUUV seroprevalence could be inversely correlated to the abundance of non-reservoir species (e.g. Apodemus species), as found in [15]. In addition, we did see any evidence that a dilution effect was occurring in the biological reserve FRFGLA and reducing the transmission of PUUV between bank voles. There are several potential explanations. Here are two plausible ones: (i) The two sites surveyed (FRFMIG and FRFGLA) might not be contrasted enough in terms of small mammal or vertebrate community to reveal a pattern of dilution effect. In fact, the biological reserve FRFGLA is relatively small, highly frequented by humans, and there might be only weak impact on animal communities in this protected forest. (ii) Some studies have shown that the dilution effect could only be observed in spring when populations are composed of breeding individuals, so that the presence of other species impacts bank voles (reduction of intraspecific encounter rates…), and not in September, when animals are non-breeding and more docile (see [32]). In this case, our dataset might be too restricted to test this hypothesis (only one spring sampling session in the biological reserve, due to ethical constraints). A deepened investigation of dilution effect patterns and underlying mechanisms is required, with an analysis of rodent species' absolute abundance, bank vole population genetics and behaviour, as well as the study of larger mammals, particularly predators, which could also impact PUUV transmission between bank voles [40]. This is critical to the future designing of effective preventive measures.

5. Conclusions

The 2021 NE epidemic in the French Jura mountains is correlated with increased bank vole abundance, and the concomitant increase in circulation and spread of PUUV in its reservoir populations, without any noticeable genetic viral change. This phenomenon was even more pronounced in the managed forest of

Mignovillard. The exceptionally high levels of PUUV seroprevalence observed from spring 2021, as well as the absence of spatial pattern detected among trap lines, reveal that a large geographic area can become infected within a few months, even if during the previous autumn, PUUV distribution had been patchy and reduced. Such results underscore the need for collaborative efforts between physicians/health departments and academic researchers to elucidate both the human and ecological aspects of such outbreaks, from the monitoring of reservoir populations and PUUV circulation in PUUV endemic areas to the design of prevention strategies when the exposure risk becomes non-negligible.

Author contributions

All authors agreed to the final version of the manuscript and its submission for publication.

Marie Bouilloud, Julien Pradel and Nathalie Charbonnel lead the field work.

Guillaume Castel performed the phylogenetic and statistical analyses.

Guillaume Castel and Nathalie Charbonnel formulated the research question and drafted the manuscript.

Lara Dutra and Hussein Alburkat performed the serological analyses.

Caroline Tatard and Mathilde Criado performed the PUUV sequencing.

Tarja Sironen and Nathalie Charbonnel were responsible of the project administration and supervision.

All authors were involved in the writing – review & editing.

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.

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Data accessibility

Raw data have been deposited in Zenodo (10.5281/zenodo.8177974).

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.idnow.2023.104767.

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