

## SHORT REPORT

# Spatial and temporal patterning of bank vole demography and the epidemiology of the Puumala hantavirus in northeastern France

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## SUMMARY

Epidemiological data from bank voles, *Myodes glareolus*, naturally infected by the hantavirus Puumala (PUUV) were collected by a capture–mark–recapture protocol from 2000 to 2002 in the French department of Ardennes. Four monitored trapping sites were established in two forests located in two cantons (Flize and Monthermé). We captured 912 bank voles corresponding to 557 different individuals during 8820 trapping nights for an overall trapping success of 10·34%. The average PUUV seroprevalence was 22·4%. Characteristics of the system reported in North European countries are confirmed in France. PUUV seroprevalence and abundance of rodents appeared weakly linked. Adult voles were more frequently antibody-positive, but no difference between sexes was established. Anti-PUUV seropositive voles were captured and high seroprevalence was observed from both forests, without human infection reported in Flize canton during the study. One site among the four exhibited peculiar infection dynamics, where vole weight and infection risk were negatively correlated.

Haemorrhagic fever with renal syndrome (HFRS) is a human disease that occurs in Europe and Asia. High fever and renal dysfunction characterize the disease [1]. This rodent-borne zoonotic infection is mainly transmitted to humans through inhalation of contaminated aerosols from the urine, saliva or faeces of infectious rodents [1]. Distinct viruses in the genus *Hantavirus*, family Bunyviridae, cause HFRS of

varying severity [1]. Nephropathia epidemica (NE) is the mildest form of HFRS, which prevails in Europe [1]. The hantavirus Puumala (PUUV) is the aetiological agent of this disease and it was isolated from a bank vole [*Myodes (Clethrionomys) glareolus*] in Finland in 1980 [1]. During the last decade, the number of human infections by PUUV has increased in Europe [1, 2]. Bank voles are the reservoir hosts [3] and their infection by PUUV usually appears without deleterious impact on fecundity or survival [3], however, Kallio [4] reported a negative effect of PUUV infection on bank vole survival over winter.

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In Europe and particularly in France the geographical distribution of bank voles is wider than the distribution of NE [1]. France presents, endemic, sporadic cases and disease-free areas, making it a critical location for the study of PUUV dynamics. The main HFRS endemic area is the Ardennes region but other areas also are affected [2]. Five major outbreaks of human NE cases have been reported since the 1990s: 1993, 1996, 1999, 2003 and 2005 [2]; and a peak in 2001 in the Ardennes [1]. The Ardennes region accounted for 30–40% of French cases of NE [5]. Since 1997, epidemiological investigations carried out in the forests of the Ardennes region have attested to the presence of PUUV and also an irregular seroprevalence within bank vole populations, according to the year [6]. However, very little is known about the spatial and temporal variability in the distribution of infected bank voles in France. Thus, characterizing hantavirus epidemiology in voles would be a preliminary step in assessing the risk of PUUV transmission to humans.

Two forests, 30 km apart, were monitored through capture–mark–recapture (CMR) studies, from 2000 to 2002. In order to identify differences in rodents and PUUV dynamics, the spruce forest of Croix-Scaille was selected because several human cases had recently been detected in its vicinity, and also the broad-leaved forest of Elan in an area where no NE cases had occurred [5]. The human cumulated incidences were 41·20 and 3·98/10 000 from 1990 to 1999 in both cantons, Monthermé and Flize respectively, where the forests are located (Augot *et al.*, personal communication). We defined two trapping sites in each forest: sites A and B in Elan forest 2 km apart, and sites C and D in Croix-Scaille forest 5 km apart. Except for site A where it is very dense, ground vegetation is rather sparse in the other three trapping sites, especially in Croix-Scaille spruce forest. We organized five trapping sessions per year in April, June, July, September and October. At each trapping site, we constructed a 7 × 7 open grid of 49 live traps. We used baited Ugglan traps, allowing multiple captures, spaced at 15-m intervals. The effective trapping area corresponded to a square of 1·1 hectares (ha). We examined the traps each morning for 3 days and handled trapped individuals. We identified the rodent species and collected a blood sample from the retro-orbital sinus after anaesthesia. We sexed and weighed each animal which was then released at its place of capture. Date, clipping code and the trap location were also recorded. Blood was stored at 4 °C until

transported to the laboratory for serological analyses. Blood samples were taken from all captured rodents and were analysed to detect antibodies against PUUV. Sera were tested for the presence of PUUV-specific IgG antibodies by indirect immunofluorescence and by enzyme-linked immunosorbent assay according to published protocols [7]. We investigated the influence of individual parameters, such as body mass or sex, and environmental parameters, such as sampling site or season of capture, on the probability of infection.

To estimate population size and seroprevalence for a given session at each site, we only considered the first capture of rodents that were trapped several times, which may occur since the trapping sessions lasted for three nights. We estimated the seroprevalence of PUUV as the proportion of infected individuals. For annual analyses, only one capture per year for each individual was taken into account. The annual seroprevalence was computed considering a rodent to be antibody-negative at first capture, then subsequently antibody-positive during the same year; or a rodent to be positive at first capture then seropositive for the entire year. Finally, for analyses of recapture data, rodents that died during their first handling were removed from the dataset.

We considered three periods: just after winter (in April, period W), the reproductive period (from June to September, period R), and the non-reproductive period (in October, period N). We studied the risk factors of infection at both individual and population levels. We defined three weight classes, corresponding to juveniles (<14·5 g), subadults (14·5–19·0 g) and adults (>19·0 g), adapted from previous field studies [8].

Statistical analyses were carried out using the R free software [9]. Analyses employed included the binomial test,  $\chi^2$  test and Student's *t* test.

We first focused on the main features at a regional scale derived from data gathered from all four sites, before detailing the particular pattern for each site. Between April 2000 and October 2002, bank voles accounted for 79% of captured small mammals. The overall density of *Myodes glareolus* populations over the two forests averaged 18·4 animals/ha in spring (April), and increased to 39·54, then 62·5 animals/ha in June and July respectively. In September and October, the density decreased (49·31 and 30 animals/ha respectively). The amplitude of seasonal variations in numbers of captured bank voles differed between trapping sites, but exhibited the same pattern. During

the study period, 536 different bank voles (497 alive and 39 dead in traps at first capture) were captured over the four sites during 8820 trapping nights. For annual analyses, we considered 557 rodents: 497 live-captured voles caught only during a single year and 60 further voles, which were caught over more than one year and were taken into account once per year of capture. Overall 376 recaptures of bank voles marked during previous trapping sessions occurred. This brought the total number of captured bank voles to 912, considering only one capture per session, giving an overall trapping success of 10.34% (15.4% in Elan forest with 679 captures, and 5.29% in Croix-Scaille forest with 233 captures). Of the 912 captured bank voles, we sexed 899 and weighed 881 individuals.

A bias in sex ratio towards males was seen, with males accounting for nearly 60% of captures (binomial test of the probability 0.5,  $P < 10^{-4}$ ) (Table 1). This sex bias towards males was seen in all sites ( $\chi^2 = 2.69$ , D.F. = 3,  $P = 0.44$ ). No relationship between sex and the probability of recapture ( $\chi^2 = 1.25$ ,  $P = 0.26$ ) nor between sex and age group ( $\chi^2 = 1.11$ ,  $P = 0.57$ ) was seen. More males than females were captured throughout the year, but the bias was only significant during the reproductive season [binomial test of the probability 0.5: (i) period W, 44.4% of females (59/133),  $P = 0.22$ ; (ii) period R, 39% (267/685),  $P < 10^{-4}$ ; (iii) period N, 44.4% (36/81),  $P = 0.37$ ]. The probability of recapture varied between seasons ( $\chi^2 = 49$ , D.F. = 2,  $P < 10^{-4}$ ) but not in the anticipated manner: the rate of recapture was high at 64% in October, after the reproductive season, but was very low at 16% just after winter.

The distribution of captured bank voles in age groups (Table 1) differed between trapping sites ( $\chi^2 = 35.6$ , D.F. = 6,  $P < 10^{-4}$ ). This fact could be linked to higher survival or lower dispersal in some sites, since the probability of recapture also differed ( $\chi^2 = 14$ , D.F. = 2,  $P = 3 \times 10^{-3}$ ). The recapture rate in April ranged from 0.1 at site A to 0.38 at site B. It was 0.22 at site C. No recaptures occurred at site D in April.

*Elan site A.* The mean number of captures per trapping session was 27.5 over the 3 years. The annual mean number of captures per session was rather high and stable, ranging from 21 in 2002 to 33 in 2001. Despite such high population levels, the proportion of young voles appeared to be low (Table 1: combined, juveniles and subadults did not represent a third of the population). During the 3 years of this study, 231 bank voles were marked and released. The number of

captures per vole ranged from one to six (on average 1.8 per trapping session). Most individuals in the population (135/231, 58.5%) were captured only once. In terms of bank vole survival, the mean number of successive trapping sessions where the 96 recaptured voles were known to have survived was 2.9 (s.d. = 0.98).

*Elan site B.* The global mean number of captures was 17.5, ranging from six in 2002 to 35 in 2001. This site had the youngest population, since the combination of juveniles and sub-adults represented over half the total population (Table 1). Over the entire study, the number of marked and released bank voles was 124. The number of captures per vole ranged from one to nine (mean of 2.1 per trapping session). The 66 recaptured individuals (53% of the marked population) were known to be alive for a mean of 3.1 trapping sessions (s.d. = 1.30). The life expectancy of voles at this site was the highest among the four studied areas, which was also the case for seropositive bank voles (survival on average for 3.8 successive sessions, s.d. = 1.71).

*Croix-Scaille site C.* The global mean number of captures was 13, ranging from four in 2000 to 23 in 2002. This site had the highest population level during 2002, a year which was less favourable to the populations in the other sites (Table 1). The proportion of adults was also high. A total of 114 different voles were caught and released here. The number of captures per vole ranged from one to eight (on average 1.8 per session, with 53.5% of the population captured only once). The 53 recaptured voles were alive on average for 2.7 trapping sessions (s.d. = 1.12).

*Croix-Scaille site D.* The global mean number of captures was very low at 2.6, and presented the largest relative fluctuations, from about 0.5 in 2000 and 2002 to seven in 2001. This site, very wet from the end of autumn to the middle of spring, seemed unable to sustain a permanent bank vole population. No voles were captured in April for any of the study years. The rate of recapture was by far the lowest. Three quarters of the captured voles in the site were adults. Few voles were caught in 2000 and 2002 on this site (Table 1). A total of 28 voles were marked and released and the number of captures per vole ranged from one to three (mean of 1.3 per trapping session). Only eight (28.6%) voles were caught repeatedly (on average 2.1 times, s.d. = 0.33).

Table 1. Distribution of antibody-positive vs. all captured bank voles for each trapping site, by sex and age (based on weight class)

Characteristics	Site A		Site B		Site C		Site D		Total	
	Positive No. (%)	Total No. (%)	Positive No. (%)	Total No. (%)	Positive No. (%)	Total No. (%)	Positive No. (%)	Total No. (%)	Positive No. (%)	Total No. (%)
<b>Sex</b>										
Male	12 (34)	247 (60)	44 (53)	149 (57)	49 (69)	117 (62)	11 (85)	24 (71)	116 (57)	537 (60)
Female	23 (66)	167 (40)	39 (47)	112 (43)	22 (31)	73 (38)	2 (15)	10 (29)	86 (43)	362 (40)
<b>Age</b>										
Juveniles	5 (14)	38 (10)	3 (3.5)	42 (16)	3 (4)	14 (7)	1 (8)	4 (11)	12 (6)	98 (11)
Subadults	8 (23)	83 (21)	23 (28.5)	91 (35)	11 (15)	44 (23)	0 (0)	6 (15)	42 (21)	224 (25)
Adults	22 (63)	270 (69)	56 (68)	127 (49)	58 (81)	134 (70)	12 (92)	28 (74)	148 (73)	559 (64)

Annual seroprevalence (numbers of antibody-positive and total captured unique bank voles for each site and year)

Year	Elan forest						Croix-Scaille forest					
	Site A			Site B			Site C			Site D		
	Pos	Total	Prev	Pos	Total	Prev	Pos	Total	Prev	Pos	Total	Prev
2000	0	95	<b>0</b>	1	38	<b>2.6</b>	5	17	<b>29.4</b>	1	1	<b>100</b>
2001	17	97 (5)	<b>17.5</b>	34	83 (1)	<b>41</b>	12	35 (1)	<b>34.3</b>	7	26	<b>27</b>
2002	6 (1)	69 (7)	<b>8.7</b>	7 (6)	19 (7)	<b>37</b>	30	74 (7)	<b>40.5</b>	0	3	<b>0</b>
<b>Total</b>												
Year	No. positive	Total no.	Mean prevalence (%)									
2000	7	151	<b>4.6</b>									
2001	70	241	<b>29</b>									
2002	43 (7)	165	<b>26</b>									

Pos, Antibody-positive captured bank voles; Total, total captured bank voles. We considered only one capture per bank vole and per year. Yearly mean prevalences (Prev) are given in bold, and numbers of antibody-positive recaptured bank voles from the previous year are given in parentheses.

Antibodies against PUUV were detected in 204 bank vole sera out of the 912 sera collected during the 3 years of study, for an overall seroprevalence of 22.4% over the four trapping sites. Antibody-positive sera came from 113 different bank voles, 59 (52.2%) of which were captured only once. On average, the recaptured fraction of the positive population remained at the trapping site for three sessions (s.d. = 1.37,  $n=54$ , range 2–9). All antibody-positive bank voles remained positive in subsequent recaptures.

In greater detail, the mean number of successive trapping sessions for seropositive recaptured bank voles were three (s.d. = 1.07,  $n=7$ ) and 3.8 (s.d. = 1.71,  $n=21$ ) in Elan forest's sites A and B respectively. In Croix-Scaille forest, 22 and four seropositive voles were recaptured in sites C and D respectively. On average, they were known to be alive for 2.4 (s.d. = 0.57) and 2.3 (s.d. = 0.43) trapping sessions respectively.

No relationship was seen between sex and infection risk (Table 1,  $\chi^2=0.076$ , D.F. = 1,  $P=0.78$ ). The global seroprevalence varied greatly depending on the year: ranging from 4.5% in 2000 to >30% in 2001 ( $\chi^2=26.2$ , D.F. = 2,  $P<10^{-4}$ ). Over the four sites, despite similar capture rates in 2000 and 2002, the estimated seroprevalence was 5–6 times higher in 2002 (Table 1, 4.6% of positive rodents in 2000 vs. 26% in 2002). The increase in antibody-positive rodents in 2002 compared to 2000 certainly resulted from the increase in mean global infection prevalence in 2001. Conversely, we did not detect a significant difference in seroprevalence between the three periods of the year (W, R, N;  $\chi^2=0.27$ , D.F. = 2,  $P=0.87$ ). Moreover, we did not find a difference between sexes in the proportion of positive adults during the autumn ( $\chi^2=0.63$ , D.F. = 1,  $P=0.43$ ). However, overall the distribution of antibody-positive bank voles appeared to be very dependent on weight class (Table 1,  $\chi^2=12.5$ , D.F. = 2,  $P=2 \times 10^{-3}$ ).

The highest number of seropositive voles were caught during 2001 but the spread of infection and demographic patterns differed between sites. The number of antibody-positive and total captured bank voles per site and year for the years 2000, 2001 and 2002 respectively, were: Elan forest (site A: 0/145, 28/166, 7/105; site B: 2/58, 67/174, 14/31); Croix-Scaille forest (site C: 7/21, 23/56, 42/117; site D: 2/2, 12/34, 0/3). Sites A and B each revealed a higher seroprevalence in 2002 than in 2000, despite a lower number of captures. We detected infected bank voles on each trapping site during several, if not all,

trapping sessions, even in the supposedly control forest of Elan.

Our study, in France, confirmed the main features reported about the PUUV–bank vole system and other hantavirus–rodent systems from European countries [3, 8]. Infection seroprevalence of the virus appeared to be higher during 2001, the year bank vole populations increased, but was not related simply to population densities [8]. The fact that seropositive voles were caught at low densities on site D, while none were caught on site A in 2000, despite high densities, contradicts a direct link between the number of captured rodents and seroprevalence. Higher numbers of seropositive bank voles were captured when the populations were decreasing from a peak year, as is the case in other hantavirus–rodent studies [10]. High bank vole densities in 2001, explains the differences in seroprevalence between 2000 and 2002 better than the immediate densities do.

The antibody-positive bank voles were mainly adults, corroborating associations between higher risks of exposure and breeding activities reported by Escutenaire *et al.* [8]. In fact, the unbalanced sex ratio observed in the four trapping sites most probably resulted from different mobility between male and female voles during the breeding period. This confirms a behavioural difference that would result in differing risks of exposure to PUUV. In fact, female territories are smaller than those of males, especially during the reproductive season [11], and males are much more tolerant of territorial overlap. Hence, more non-resident males than females would be captured on the trapping grids. However, contrary to some other studies [3], no relationship was seen between sex and infection risk, which may be due to insufficient data.

Our study produced some surprising observations. First, Elan forest, which was our control forest, sustained PUUV-infected bank vole populations, even at high seroprevalence (particularly site B). Second, an important turnover in vole populations appeared between the trapping sessions in October and the following one in April. This observation suggests that in addition to potentially high mortality, possibly linked to the infective status [4], a significant dispersal of bank voles occurs during the non-reproductive season or at its onset due to breeding behaviour. The lowest mean recapture rate in April was reported for site A (10.2% on average), although it appeared to be the most favourable to the bank vole population. Some bank voles appeared to be long-lived, such as in



site B where an antibody-positive vole was repeatedly captured from June 2001 to October 2002. Therefore, other factors beyond demography, may modulate the dynamics of PUUV in France.

Most human infections occur during late spring and summer [1] when the bank vole breeding season is at its peak. Occasional mast years (heavy seed crops of oak and beech) lead to pullulations of seed-eating rodents like the bank vole. The mastings can be synchronous over large areas, resulting in human hantavirus epidemics. In fact, after the masting in the previous autumn, winter survival of rodents is good and rodents start to breed earlier than during normal years, bringing high densities early in summer [1, 6]. This high density and the continuous recruitment of susceptible rodents from the newborn population maintains a high level of PUUV circulation [3, 8], making the breeding season, and the summer in particular, the most risky period for humans.

Understanding the influence of habitat heterogeneities on the risk of infection appears to be of crucial importance. Bank vole demography presented great variability between proximal trapping sites. Significant differences in infection dynamics were also observed between sites, as reported in other studies on the bank vole–PUUV system [8]. Trapping site A, in the forest of Elan, presented an original pattern as far as infection dynamics was concerned. This site presented the best conditions for high transmission of PUUV: the population exhibited a large increase between spring and summer of each year and bank vole densities were the highest of the four trapping sites. The virus was present in the population but the seroprevalence remained low. In addition, a postulated link between the age of the rodent and the risk of infection was not upheld here. In future studies, we will focus on the impact of habitat heterogeneities in order to better understand the mechanisms of virus transmission.

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#### DECLARATION OF INTEREST

None.

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