Examensarbete
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# Local habitat choice of Puumala virus (PUUV) seropositive bank voles (*Myodes glareolus*) in Northern Sweden

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## Abstract
In humans, Puumala virus (PUUV) of the genus *Hantavirus* causes hemorrhagic fever with renal syndrome. The primary host-reservoir in Europe is the bank vole (*Myodes glareolus*). To understand the persistence of PUUV among hosts we must understand the environment that the bank vole prefers, and therefore, the environment in which PUUV is harboured. In this study bank voles in Västerbotten, Sweden were sampled in 1998 according to documented human infections occurring at different sites between the years of 1991 to 1996, and at paired random control sites. It was confirmed that bank vole abundances, irrespective of PUUV status, were higher in sites located in highly productive, old spruce (*Picea abies*) forests with a dominating undergrowth of bilberry (*Vaccinium myrtillus*). I suggest that, in order to better predict the outbreaks of PUUV, we need a better understanding of the population dynamics of the bank vole and individual vole behaviour with respect to the differences in habitat choice at a regional scale.

## Introduction
The ecology of zoonotic diseases is emerging as a pressing issue as these diseases are finding their way into human populations. Puumala virus (PUUV), of the genus *Hantavirus*, is one such causative agent; causing Nephropathia epidemica (NE), a mild hemorrhagic fever with renal syndrome (HFRS) in humans (Yanagihara et al. 1985). The primary host of PUUV in Europe is the bank vole (*Myodes glareolus*) (Brummer-Korvenkontio 1980). Puumala virus infects humans predominantly through the inhalation of virus-contaminated aerosols of rodent excretion (Bernshein et al. 1999, Schmaljohn and Hjelle 1997).

It has been suggested that the incidence rate of NE displays a similar pattern to that of the PUUV reservoir-hosting bank vole population dynamics in Northern Sweden (Olsson et al. 2003, Hörnfeldt 1994). With reference to the population dynamics, peak intervals of small mammals seem to surface approximately every three years (Olsson et al. 2002, Hörnfeldt 1994). As an omnivorous, habitat generalist, the bank vole is found in a vast number of environments throughout the Eurasian continent. Although the bank vole is found in all areas of Sweden, except the high altitudes of the northern mountain range, most cases of human NE infection are found in the northern counties. If we want to limit the incidence rate of NE it is vital to understand the ecology of bank voles in relation to the epidemiology of PUUV and NE.

Demographic studies on the bank vole have shown that there is a correlation between the time of year, animal sex, age and the number of seropositive animals captured (Olsson et al. 2002, Bernshein et al. 1999). Most cases of PUUV seropositive animals were caught in late summer or early fall, suggesting that PUUV is transmitted primarily during the season of high reproduction (Bernshein et al. 1999). Several studies have found that adult males seem to carry PUUV antibodies more frequently then females as they are more motile during the mating season and may participate in more aggressive behaviour when compared to female voles, passing PUUV from animal to animal horizontally (Olsson et al. 2002). Infected female bank voles transfer maternal PUUV antibodies to progeny, giving raise to seemingly PUUV infected, but actually juvenile specimens protected against horizontal PUUV infection (Kallio et al. 2006). A juvenile inheriting its mother's antibodies will clear the antibodies after, approximately, 3 months, and is then susceptible to an infection (Kallio et al. 2006). Olsson et al. (2002) found that the demographic factor with the highest influence on the probability of PUUV infected bank voles was age. The older the bank vole is, the higher the chance of having been exposed to PUUV.

It has been suggested that many infectious wildlife diseases may pursue meta-population dynamics, meaning that higher local densities of the host animals would act as a source for the disease on a regional scale (Grenfell and Harwood 1997, Hess et al. 2002). Higher bank vole densities are associated with adequate food supply and extensive shelter from predators (Olsson et al. 2003, 2005**,** Hansson et al. 2000). *Vaccinium myrtillus* (berries, leaves and twigs), *Melampyrum pretense* (seeds) and *Alectoria* spp. (hanging lichens) provide some of the food items consumed by bank voles (Viro and Sulkava 1985). Shelter is provided by dead wood, standing and fallen (Tallmon and Mills 1994, Ecke et al. 2002). Together, food availability and shelter largely determine the bank vole habitat selection (Viro and Sulkava 1985, Tallmon and Mills 1994, Ecke et al. 2002).

In one study by Olsson et al. (2005) it was found that there were positive correlations between the number of bank voles and moist *Picea abies* forests with a substantial amount of herbaceous undergrowth, and a negative correlation with *Pinus sylvestris* forests which are commonly associated with drier and nutrient poor undergrowth. The relevance of habitat composition on successful hantavirus persistence and circulation among rodent host populations renders increasing interest (Linard et al. 2007). It seems that hanta viruses persist in sparse host populations in restricted "hot zones" of favourable habitat composition to their hosts (Abbott 1999).

In the present study, I will also evaluate the importance of different environmental factors, but in contrary to Olsson et al. (2005), I will include all available habitat factor data sampled on a local scale, to determine where the distribution of PUUV positive bank voles lies with respect to bank vole habitat choice. Subsequently I will attempt to answer the following questions:

- **Do PUUV seropositive bank voles prefer a specific habitat type with respect to different environmental factors?**
- **Which types of environmental factors determine the distribution of PUUV seropositive bank voles within a highly endemic region?**
- **Do juvenile bank vole individuals, who have inherited their mother's antibody response to PUUV, dilute or amplify the signal in the habitat type in which PUUV positive bank voles are found?**

## Materials and Methods

### Study sites and animal sampling

The sampling of voles was carried out in the fall of 1998 in the county of Västerbotten (63°08'-64°45'N:18°55'-21°00E). 101 sites within this area were identified to have patients exposed to PUUV during the interval of 1991 to 1996. The 101 patients identified the time and place of PUUV exposure with complete confidence. Randomly, 16 of these 101 sites were selected for vole trapping. For each of these case sites, a control site, with no known history of NE**,** was sampled for comparisons between bank vole numbers and habitat composition. The control sites direction in comparison to the paired case sites were selected at random between 1 and 360 degrees. Distances between case and control sites were set randomly from 1-10km. The distances were selected with the purpose to be able to identify the relevant distance with regards to the spatial scale and bank vole PUUV correlations. The distance of at least 1km was selected in order to allow for independent sampling based on the assumption that voles are moving less then the selected distances (Olsson et al. 2005, Kozakiewicz et al. 1993). In total, 32 sites were selected for bank vole trapping.

Both case (locations of exposure) and control sites were selected using maps (1:10000). Case and random sites were divided into squares of 450mx450m, where the patients dwelling and random paired position became the centre point of the square. 16 sampling positions, in a 4x4 grid, 150m apart were distributed within each square. A GPS reading was taken in the centre of each square in order to accurately identify each position. Positions falling directly on arable/cultivated lands, roads, waterbeds, or other highly unsuitable environment for small mammals, were excluded from sampling. Therefore the actual number of sampling positions for each site ranged from 11 to 16 with the exception of one site where 7 positions fell on waterbeds and the remaining 9 positions were used.

10mx10m quadrates were laid out at each sampling position in a Northing Easting direction for bank vole trapping. In the corners of each quadrate, 3 snap traps, bated with dry apple, were placed in optimal positions within 1m from each corner's flag, resulting in a total of 12 traps per quadrate (see Olsson et al 2005 for details). Traps were checked for 3 consecutive days, resulting in 36 trapping nights per quadrate. Results were expressed as the total number of voles captured per 100 trap nights (i.e. according to Hanski et al (1994) is a reflection of the relative population density on each sampling occasion). Snap-trapping took place during late September and early October. Paired sites were trapped simultaneously, and were not subject to previous bating.

Caught animals were kept on dry ice and transferred to -70°C freezers. Animals were processed in bio-safety level 3 laboratories. Total body weight was recorded to the nearest 0.1grams and weights of foetuses were subtracted. Blood was collected using Nobuto blood filter strips (Toyo Roshi Kaisha, Ltd., Tokyo Japan) and dried for later processing. Strips were eluated with 500µl of phosphate-buffered saline (PBS) at room temperature for 1 hour. The dilution was estimated at 1:12.5. The presence of PUUV infection was determined by detecting the anti-PUUV immunoglobulin G immunosorbent assay (ELISA) (for results see Appendix 3) according to previous protocols (Elgh et al. 1996, Ahlm et al. 1997).

**Habitat sampling**

Olsson et al. (2005) sampled vegetation and habitat factors according to Riksskogstaxeringen (1995). The data was sampled in this way so that if some factors were found to influence the distribution of PUUV positive voles it would be possible to use data collected in previous years to determine where puumala virus may be distributed throughout Sweden. Of the 16 site pairs used for vole sampling, 7 pairs were randomly selected for habitat characteristics. During the summer of 1999 habitat features were recorded for each small quadrate, see appendix 1a and b. Within a 10m radius of the centre of each square, characteristics of ground, soil and tree composition were sampled on a nominal level. Within a 0.25$m^2$ quadrate at each corner centre of the 10mx10m small quadrates coverage of herbs, grasses and bryophytes were recorded. These 5 samples were converted to a mean number for the entire quadrate. Ground cover of the vegetative species was assigned to 1 of 7 different classes of coverage: 0%, 1-5%, 6-20%, 21-40%, 41-60%, 61-80% and >80%.

**Statistical analysis**

All of the vegetative and other habitat factors data were assessed in the statistical analysis in order to determine if one or several factors were contributing to the habitat choice or

vole distribution of PUUV seropositive voles. The vegetative data was analyzed using a principle component analysis (pca) as the data was multivariate. The pca was repeated three times, firstly, including only the species data, secondly, only the other habitat factors and thirdly, including both the plant species and the habitat factors. With this analysis complete it was possible to compare the number of seropositive animals with the vegetative analysis using a general linear mixed model with normal random effects using Penalized Quasi-likelihood (Schall 1991, Breslow and Clayton 1993, Wolfinger and O'Connell 1993, Venables and Ripely 2002) using the Site as an error term to compare the relationship between the voles and the different components from the pca analysis. Adult voles and juvenile voles were assessed separately and together. According to Bujalska and Gliwicz (1972) sexually mature (adult) female bank voles have been estimated to weigh between 17.1 ± 0.3g and 25.2 ± 0.5g, where as sexually immature (juvenile) female bank voles can weigh between 12.8 ± 0.1g and 18.2 ± 0.1g. As sexually mature females weigh more then sexually mature males, and juveniles do not seem to show a difference in weight between the sexes (Pankakoski and Tähkä 1982) I used female weight to describe adult bank voles in my study. I have chosen to describe adult bank voles as weighing more then 17.99g in my data in order to eliminate as many juveniles as possible, while still maintaining as many adults as possible in each of the samples.

The small quadrates were not assessed as they did not explain the variance in the hierarchy of the data set.

Correlation tests were done to produce the arrows shown on the figures (figure 1 and 2), however it was not possible to trust the p-values simulated by the correlations as the correlations are designed for parametric tests and the analysis ran was much more complicated, combining multivariate data with a general linear mixed model with normal random effects using a Penalized Quasi-likelihood. However it is justifiable to trust the correlation directional values and pictures the correlations produce in the figures.

## Results

Of the 2 173 bank voles collected in 1998, 17.6% were determined to be PUUV seropositive (Olsson et al. 2005).The three principal component analysis (pca) showed that the first two components accounted for 22.6%(vegetation only) of the variance, 33.3% (habitat factors only) of the variance and 18.7% (vegetation and habitat factors) of the variance (see table 1). This weak output from the different components explaining the variation in the data is due to the number of variables tested in each pca: the more variables going into the pca, the more theoretical components coming out of the pca but the more variables going into a pca the weaker each component becomes.

Table 1. The first 6 principal components (2a-2c) for each of the pca analysis showing what amount of variation in the data is explained by each component: 2a shows the vegetation only pca, 2b shows only the habitat factors only pca and 2c shows both the vegetation and the habitat factors pca together.

| 2a<br>Importance of components: | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 | Comp. 5 | Comp. 6 |
|---|---|---|---|---|---|---|
| Standard deviation | 1.776 | 1.508 | 1.224 | 1.180 | 1.152 | 1.137 |
| Proportion of variance | 0.131 | 0.095 | 0.062 | 0.058 | 0.055 | 0.054 |
| Cumulative proportion | 0.131 | 0.226 | 0.289 | 0.347 | 0.402 | 0.456 |

| 2b<br>Importance of components: | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 | Comp. 5 | Comp. 6 |
|---|---|---|---|---|---|---|
| Standard deviation | 2.283 | 1.855 | 1.431 | 1.348 | 1.303 | 1.166 |
| Proportion of variance | 0.201 | 0.132 | 0.0788 | 0.069 | 0.065 | 0.052 |
| Cumulative proportion | 0.201 | 0.333 | 0.411 | 0.481 | 0.547 | 0.599 |

| 2c<br>Importance of components: | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 | Comp. 5 | Comp. 6 |
|---|---|---|---|---|---|---|
| Standard deviation | 2.310 | 2.041 | 1.689 | 1.534 | 1.508 | 1.446 |
| Proportion of variance | 0.105 | 0.082 | 0.0560 | 0.0462 | 0.044 | 0.041 |
| Cumulative proportion | 0.105 | 0.186 | 0.242 | 0.288 | 0.333 | 0.374 |

Seropositive adult bank voles were positively correlated with both component one (DF=176, p-value=0.0084) and two (DF=176, p-value=0.0275) but were not positively correlated with the interaction between components one and two (DF=176, p-value=0.2766) from the pca ran only with the vegetative species analysis. Seropositive juvenile bank voles were positively correlated with component one (DF=176, p-value=0.0093), however, they showed no significant correlation with component two (DF=176, p-value=0.1031) or the interaction between component one and two (DF=176, p-value=0.9083). The total number of seropositive bank voles (both adults and juveniles) show a significant correlation with component one (DF=175, p-value=0.0010), and component two (DF=175, p-value=0.0128) but show no significance when compared to the interaction between the components (DF=175, p-value=0.5382) (Figure 1) (For summary see Table 2).

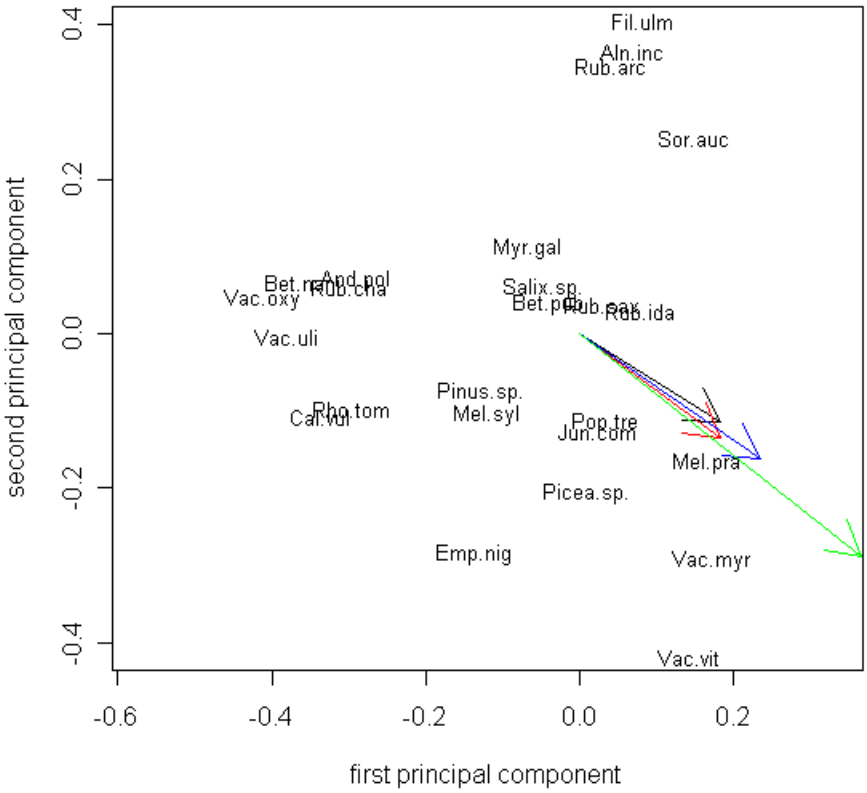


Figure 1. The principal component analysis showing the plant species of the different sites and their relationship to the seropositive voles (pca includes only vegetative species). The species are each represented by the fist three letters of the genus name and the first three letters of the species name, see Appendix 1a for full names. The total number of bank voles is represented by a green arrow. The total number of seropositive bank voles is represented by a blue arrow. The seropositive adult bank voles are represented with a black arrow; seropositive juveniles are represented with a red arrow. Arrows for the different vole classes were designed using the values from a correlation test between seropositive voles and the different components.

When compared only with the habitat factors adult seropositive bank voles showed a positive correlation with component one (DF=176, p-value=0.0274) but no significant correlation with component two (DF=176, p-value=0.2527) or the interaction between component one and two (DF=176, p-value=0.8697). Seropositive juveniles showed no significant correlations when compared with the different components (Component one=DF=176, p-value=0.1742; Component two=DF=176, p-value=0.5938; interaction between component one and component two=DF=176, p-value=0.0520). The total number of seropositive bank voles showed no significant correlations when compared with component one (DF=175, p-value=0.8411), component two (DF=175, p-value=0.5162) and the interaction between the components (DF=175, p-value=0.1901) (Figure 2) (For summary see Table 2).

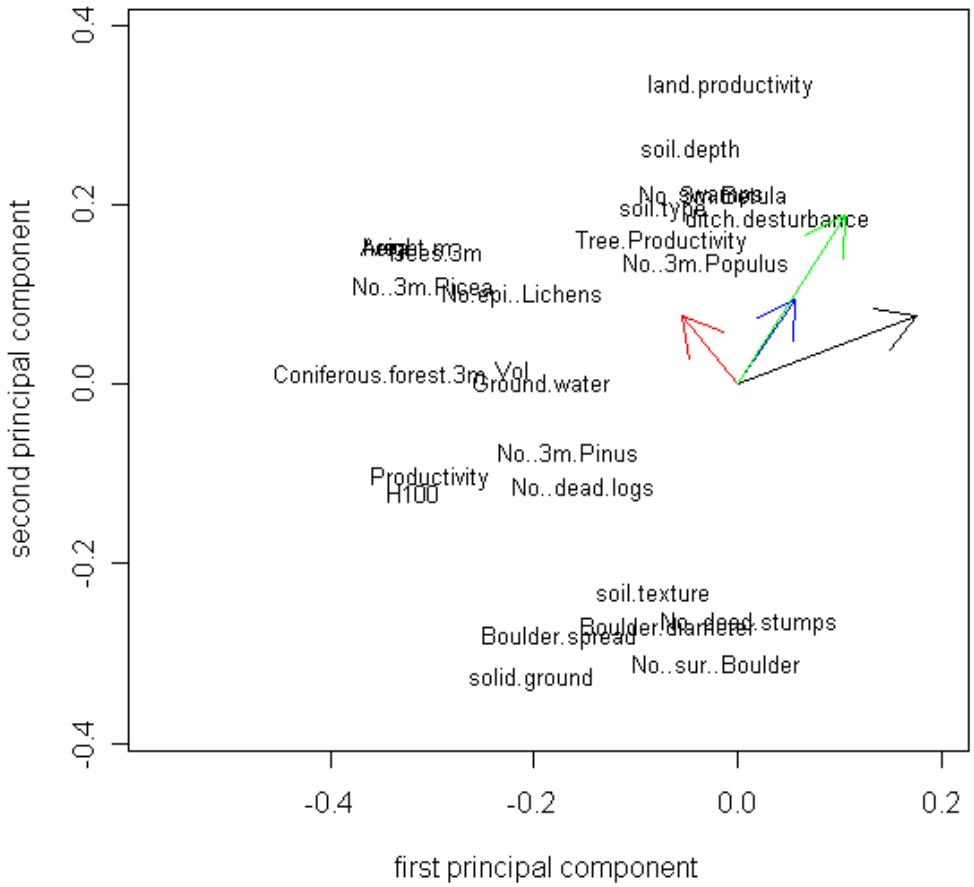


Figure 2. The principal component analysis showing only the habitat factors of the different sites and their relationship to the seropositive voles (pca includes only habitat factors) (see Appendix 1b for full names). The total number of bank voles is represented by a green arrow. The total number of seropositive bank voles is represented by a blue arrow. The seropositive adult bank voles are represented with a black arrow; seropositive juveniles are represented with a red arrow. Arrows for the different vole classes were designed using the values from a correlation test between seropositive voles and the different components.

When the pca was run as a whole, taking into consideration both the vegetation (plant species) and the habitat factors seropositive adults have significant correlations when compared to both component one (DF=176, p-value=0.0070) and component two (DF=176, p-value=0.0061), but there is no significant interaction when compared with the interaction between component one and two (DF=176, p-value=0.4624). Seropositive juveniles show no significant correlations when compared with component one (DF=176, p-value=0.4367) but do show a significant correlation when compared with component two (DF=176, p-value=0.0338). Juveniles show no significance correlation when compared to the interaction between the components, one and two (DF=176, p-value=0.6600). The total number of seropositive bank voles show no significant correlation when compared with component one (DF=175, p-value=0.4275) but do show a significant correlation when compared with component two (DF=175, p-value=0.0081) and no significant correlation when compared with the interaction between components (DF=175, p-value=0.9895) (For summary see Table 2).

Table 2. Summary of results from all three pca's, showing significant or non-significant results for seropositive adult, juvenile and the total number of seropositive bank voles. None of the tests yielded significant results for the interaction between component one and two, therefore, the results from the interaction are not summarized in the table. Comp = component. P-values ≤0.05 are significant.

| | **Vegetation only** | | **Habitat factors only** | | **Vegetation and habitat factors** | |
|---|---|---|---|---|---|---|
| | **Comp. 1** | **Comp. 2** | **Comp. 1** | **Comp. 2** | **Comp. 1** | **Comp. 2** |
| **Seropositive adults** | p-value= 0.008 | p-value= 0.028 | p-value= 0.027 | p-value= 0.253 | p-value= 0.007 | p-value= 0.006 |
| **Seropositive juveniles** | p-value= 0.009 | p-value= 0.103 | p-value= 0.174 | p-value= 0.594 | p-value= 0.437 | p-vlaue= 0.034 |
| **Total seropositive** | p-value= 0.001 | p-value= 0.013 | p-value= 0.841 | p-value= 0.516 | p-value= 0.428 | p-value= 0.008 |

## Discussion

Seropositive bank voles and seronegative bank voles seem to prefer the same habitat type, meaning that individuals, irrespective of PUUV status, have a tendency to segregate towards more productive forest habitat type. Similar observations were described by Deter et al. (2007) where they looked at the relatedness and distributions of bank voles in relation to seropositive individuals. They concluded that relatedness between the bank voles was important for distribution and whether or not the vole was seropositive but acknowledged that the distribution of the virus seemed to be random within the study areas.

Similarly, to Olsson et al. (2005) who looked at habitat factors associated with bank voles and hantavirus in Northern Sweden, I found that higher numbers of bank voles were found in areas with preferred food items (*Vaccinium myrtillus, Melampyrum pratense*) (Viro and Sulkava 1985) which seem to be associated with the shelter of spruce (*Picea abies*) forests. However, in contrast to Olsson et al. (2005), I did not find that there was a significant correlation between the numbers of bank voles and objects which may provide shelter, such as tree stumps and fallen wood (Tallmon and Mills 1994, Ecke et al. 2002).

My findings suggest that seropositive bank voles do not seem to behave differently, with respect to habitat choice, than seronegative bank voles. There is, however, a definite pattern of habitat choice among bank voles (irrespective of PUUV status) at the landscape level. I have shown (see figure 1 and 2) that bank voles choose their habitat with respect to the vegetation, in my case species of plants, as opposed to the other factors found in the local habitat (see Table 2 and Appendix 2a-2c). To look further into the idea of local habitat choice and behaviour of PUUV seropositive bank voles it might be an idea to follow up the current study by tracking a high number of individual bank voles both seropositive and seronegative to see if the behaviour of the voles is actually different with regards to local habitat choice.

On the whole, I found no evidence of a dilution or amplification effect of the seropositive juvenile bank voles in comparison to the total number of seropositive bank voles when compared to the vegetative factors (plant species only). Both seropositive juvenile bank voles and seropositive adult bank voles seem to prefer spruce forest habitat or the vegetation found there in, meaning that both juveniles and adults have a tendency to segregate towards more productive forest habitat type. However, I did find a slight dilution effect of the seropositive juveniles with respect to component one when compared

with the habitat factors, as well as with the combined analysis of the vegetation and habitat factors. This dilution seems as though it may have been enough to dilute the total number of seropositive bank voles with respect to component one.

**Conclusion**

To be able to more accurately forecast the occurrence of high numbers of PUUV seropositive bank voles we must be able to better predict the peaks and crashes of the bank vole cycles (see Olsson et al. 2007). The higher abundance of bank voles there is in a local habitat the higher the likelihood of an increase in PUUV positive bank voles, which, in turn, may increase the likelihood of more human infections. For example, 1998 was a peak year for bank vole populations in Sweden (Olsson et al. 2002, Hörnfeldt 1994) and accordingly, there were also a record number of human PUUV infections reported in Sweden that year (Olsson et al. 2003). Since the true incidence, including sub-clinical infections, may be 7-8 times higher then reported , it is suggested that as many as 4500 people in the northern counties of Sweden may have been infected by PUUV in 1998 (Olsson et al. 2003).

Despite that occasionally high local densities of the carrier, bank vole, are found throughout Sweden, 90% of all NE cases have been reported in the 4 northern most counties (Olsson et al. 2002, 2003, 2007). This may indicate that environmental properties restricted to Sweden north of approximately N60 deg Lat, other then vole densities, could contribute to the presence and abundance of PUUV positive bank voles. Therefore, further studies must be carried out to evaluate the habitat type of the more southern bank vole regions in Sweden in order to reveal larger regional patterns of seropositive bank vole abundances. Studies should, furthermore, include comparisons on a small scale, small quadrate to small quadrate, as well as between-region comparisons, to evaluate the importance of habitat factors for the occurrence of PUUV positive bank voles.

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Appendix 1a: A list of sampled vegetation in the present study. These species were sampled to determine what the specific local habitat of PUUV seropositive individuals is comprised of and which vegetative species, if any, are more important for seropositive individuals in habitat choice.

| Vegetation scientific name | Abbreviation | Reason for sampling | Type of data |
|---|---|---|---|
| Alnus incana | Aln.inc | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Andromeda polifolia | And.pol | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Betula nana | Bet.nan | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Betula pubescens | Bet.pub | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Calluna vulgaris | Cal.vul | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Empetrum nigrum | Emp.nig | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Filipendula ulmaria | Fil.ulm | info about food availability (*4) | Categorical |
| Juniperus communis | Jun.com | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Melampyrum pratense | Mel.pra | food item (*5) | Categorical |
| Melampyrum sylvaticum | Mel.syl | food item (*5) | Categorical |
| Myrica gale | Myr.gal | info about soil nutrience and habitat preference, possible food item (*1) | Categorical |
| Picea abies | Picea sp. | info about forest type and habitat preference | Categorical |
| Pinus sylvestris | Pinus sp. | info about forest type and habitat preference | Categorical |
| Populus tremula | Pop.tre | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Rhodoendron tomentosum | Rho.tom | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Rubus arcticus | Rub.arc | info about soil nutrience and habitat preference, possible food item (*1) | Categorical |
| Rubus chamaemorus | Rub.cha | info about soil nutrience and habitat preference, possible food item (*1) | Categorical |
| Rubus idaeus | Rub.ida | info about soil nutrience and habitat preference, possible food item (*1) | Categorical |
| Rubus saxatilis | Rub.sax | info about soil nutrience and habitat preference, possible food item (*1) | Categorical |
| Salix sp. | Salix sp. | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Sorbus aucuparia glabrata | Sor.auc | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Vaccinium myrtillus | Vac.myr | Food item (*4; *5) | Categorical |
| Vaccinium oxycoccos | Vac.oxy | info about soil nutrience and habitat preference, possible food item (*1; *2) | Categorical |
| Vaccinium uliginosum | Vac.uli | info about soil nutrience and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |
| Vaccinium vitis-idaea | Vac.vit | info about soil nutrience/moisture and habitat preference, possible food item (*1; *2) or shelter from predators (*3) | Categorical |

*1=Moen et al. 1993; 2= Hansson 1993; 3 = Batzali 1992; 4 = Hjältén et al. 1996; 5 = Viro and Sulkava 1985

Appendix 1b: A list of sampled habitat characteristics in the present study. These habitat factors were sampled to determine what the specific local habitat of PUUV seropositive individuals is comprised of and which factors, if any, are more important for seropositive individuals in habitat choice. Habitat factors were sampled according to Riksskogstaxeringen (1995).

| **Habitat factors name** | **Abbreviation** | **Reason for sampling** | **Type of data** |
|---|---|---|---|
| Tree Productivity | Tree Productivity | info about shelter availability and forest type | |
| Number of >3m Pinus | No.>3m Pinus | info about forest age and height (22m. approx. height for a 100yr. old tree (*1)) | Continuous |
| Number of >3m Picea | No.>3m Picea | info about forest age and height (22m. approx. height for a 100yr. old tree (*1)) | Continuous |
| Number of >3m Betula | No.>3m Betula | info about forest age and height (22m. approx. height for a 100yr. old tree (*1)) | Continuous |
| Number of >3m Populus | No.>3m Populus | info about forest age and height (22m. approx. height for a 100yr. old tree (*1)) | Continuous |
| Trees>3m | Trees>3m | info about forest age and height (22m. approx. height for a 100yr. old tree (*1)) | Continuous |
| Coniferous forest>3m | Coniferous forest>3m | info about forest age and height (22m. approx. height for a 100yr. old tree (*1)) | Continuous |
| Area | Area | info about shelter availability and forest age | |
| Height (m) | height m. | info about shelter availability and forest age (22m. approx. height for a 100yr. old tree (*1)) | Continuous |
| Tree Volume | Vol | info about shelter availability and forest age | |
| Overhead height and productivity index | H100 | info about shelter availability and forest age | |
| Productivity | Productivity | Total productivity measure | |
| land productivity | land productivity | info about shelter availability and habitat suitability | |
| Ground water | Ground water | info about water availability for vegetation factors | |
| ditch disturbance | ditch disturbance | info about habitat disturbance | |
| swamps | swamps | info about forest type | |
| soil type | soil type | info about soil composition and shelter (*2) | Nominal |
| soil texture | soil texture | info about soil composition and shelter (*2) | Categorical |
| soil depth | soil depth | info about soil composition | Categorical |
| Number of Boulders | No. sur. Boulder | info about shelter availability (*2) and water drainage | Continuous |
| Boulder spread | Boulder spread | info about shelter availability (*2) | |
| Boulder diameter | Boulder diameter | info about shelter availability and soil nutrience | |
| solid ground | solid ground | info about shelter availability and soil nutrience | |
| Number of epiphytic Lichens | No.epi. Lichens | info about food, shelter and nesting material (*3) | Continuous |
| Number of dead stumps | No. dead stumps | info about shelter availability (*4; *5) | Continuous |
| Number of dead logs | No. dead logs | info about shelter availability (*4; *5) | Continuous |

*1 = Barth, A. Pers. comm; 2 = Hansson 1978; 3 = Hansson 1999; 4 = Tallmon and Mills 1994; 5 = Ecke et al. 2002

Apendix 2a: Numerical results of the species only pca. Only the first six components of 26 components are shown (for full names see Apendix 1a)

| **Species** | **Comp.1** | **Comp.2** | **Comp.3** | **Comp.4** | **Comp.5** | **Comp.6** |
|---|---|---|---|---|---|---|
| Vac.myr | o.182 | -0.285 | | 0.326 | 0.312 | |
| Vac.vit | 0.147 | -0.424 | 0.297 | | | |
| Roh.tom | -0.296 | -0.107 | 0.251 | | 0.118 | -0.169 |
| Cal.vul | -0.334 | -0.113 | 0.291 | | -0.169 | |
| And.pol | -0.291 | | -0.154 | 0.131 | -0.186 | 0.208 |
| Vac.oxy | -0.416 | | | 0.188 | 0.214 | |
| Vac.uli | -0.385 | | | 0.189 | | 0.187 |
| Emp.nig | -0.134 | -0.290 | 0.430 | | -0.341 | |
| Mel.pra | 0.174 | -0.157 | -0.140 | 0.330 | 0.221 | |
| Fil.ulm | | 0.412 | 0.328 | 0.132 | | |
| Rub.cha | -0.303 | | -0.185 | 0.108 | 0.216 | 0.130 |
| Rub.arc | | 0.353 | 0.122 | | -0.141 | |
| Rub.sax | | | -0.155 | | | -0.183 |
| Picea.sp | | -0.196 | 0.175 | 0.137 | 0.118 | 0.156 |
| Pinus.sp | -0.131 | | 0.167 | -0.449 | 0.283 | |
| Jun.com | | -0.128 | | 0.308 | -0.187 | 0.156 |
| Salix.sp | | | | | -0.228 | -0.151 |
| Pop.tre | | -0.103 | | 0.307 | 0.228 | -0.151 |
| Myr.gal | | 0.103 | -0.225 | | -0.200 | |
| Bet.pub | | | -0.132 | -0.281 | | 0.526 |
| Bet.nan | -0.364 | | | | 0.298 | -0.175 |
| Aln.inc | | 0.373 | 0.425 | 0.216 | | |
| Sor.auc | 0.146 | 0.264 | 0.159 | | 0.256 | 0.176 |
| Rub.ida | | | 0.103 | -0.296 | 0.295 | 0.386 |

Apendix 2b: Numerical results of the habitat factors only pca. Only the first six components of 26 components are shown (for full names see Apendix 1b)

| **Habitat Factors** | **Comp.1** | **Comp.2** | **Comp.3** | **Comp.4** | **Comp.5** | **Comp.6** |
|---|---|---|---|---|---|---|
| Tree.Productivity | | 0.160 | 0.244 | | 0.308 | -0.141 |
| No.>3m.Pinus | -0.166 | | -0.438 | -0.313 | -0.235 | |
| No.>3m.Picea | -0.308 | 0.110 | 0.210 | 0.107 | | 0.195 |
| No.>3m.Betula | | 0.213 | | -0.370 | 0.364 | |
| No.>3m.Populus | | 0.135 | 0.178 | -0.230 | 0.266 | -0.154 |
| Trees.>3m | -0.298 | 0.150 | -0.198 | -0.395 | | |
| Coniferous forest >3m | -0.350 | | -0.224 | 0.190 | -0.196 | 0.160 |
| Area | -0.344 | 0.154 | 0.125 | | | 0.146 |
| Height.m. | -0.321 | 0.151 | 0.113 | | | |
| Vol | -0.221 | | 0.237 | | | |
| H100 | -0.300 | -0.121 | | 0.106 | -0.182 | -0.320 |
| Productivity | -0.300 | -0.103 | | 0.101 | -0.254 | -0.320 |
| Land.productivity | | 0.334 | -0.320 | 0.293 | | |
| Ground.water | -0.192 | | | | 0.147 | -0.425 |
| Ditch.disturbance | | 0.187 | -0.128 | | 0.330 | |
| swamps | | 0.209 | -0.239 | 0.396 | | 0.134 |
| Soil.type | | 0.196 | -0.383 | 0.244 | 0.110 | |
| Soil.texture | | -0.231 | -0.232 | 0.106 | 0.296 | |
| Soil.depth | | 0.261 | -0.111 | 0.174 | | -0.408 |
| No.sur.Boulder | | -0.310 | -0.148 | | 0.274 | 0.231 |
| Boulder.spread | -0.174 | -0.282 | | 0.111 | 0.287 | |
| Boulder.diameter | | -0.268 | | 0.130 | 0.181 | |
| Solid.ground | -0.202 | -0.327 | | | | 0.236 |
| No.epi.Lichens | -0.211 | | 0.251 | 0.231 | | 0.236 |
| No.dead.stumps | | -0.266 | | 0.187 | 0.179 | |
| No.dead.logs | -0.151 | -0.116 | | 0.123 | 0.145 | 0.374 |

Appendix 2c: Numerical results of the species and habitat factors combined pca. Only the first six components of 51 components are shown (for full names see Appendicies 1a,b)

| **Species** | **Comp.1** | **Comp.2** | **Comp.3** | **Comp.4** | **Comp.5** | **Comp.6** |
|---|---|---|---|---|---|---|
| Vac.myr | | 0.166 | | -0.316 | | |
| Vac.vit | | -0.125 | -0.374 | -0.119 | | |
| Roh.tom | | -0.144 | 0.247 | -0.109 | | |
| Cal.vul | | -0.213 | 0.238 | -0.111 | | |
| And.pol | | -0.185 | 0.223 | | | |
| Vac.oxy | | -0.214 | 0.351 | | | -0.125 |
| Vac.uli | | -0.210 | 0.287 | | | |
| Emp.nig | | -0.139 | | -0.252 | -0.119 | 0.126 |
| Mel.pra | | | -0.149 | -0.173 | | |
| Fil.ulm | | 0.113 | | 0.363 | | |
| Rub.cha | | -0.170 | 0.222 | | -0.122 | -0.132 |
| Rub.arc | | | | -0.122 | -0.132 | |
| Rub.sax | | | | 0.347 | | |
| Picea.sp | | | | | | |
| Pinus.sp | | | | -0.149 | -0.244 | 0.143 |
| Jun.com | | | | -0.138 | | -0.120 |
| Salix.sp | | | | | | |
| Pop.tre | | | | -0.102 | | 0.102 |
| Myr.gal | | | | | 0.172 | -0.145 |
| Bet.pub | | | | | -0.175 | -0.269 |
| Bet.nan | | -0.192 | 0.292 | | | |
| Aln.inc | | 0.106 | | 0.348 | | 0.107 |
| Sor.auc | | 0.166 | | 0.203 | | |
| Rub.ida | | | | | -0.108 | |
| Mel.syl | | -0.138 | | | -0.101 | 0.377 |
| Habitat Factors | | | | | | |
| Tree.Productivity | | | 0.167 | | 0.148 | 0.249 |
| No.>3m.Pinus | -0.166 | | | | -0.332 | -0.196 |
| No.>3m.Picea | -0.298 | 0.131 | | | 0.203 | |
| No.>3m.Betula | | 0.149 | 0.155 | | | 0.346 |
| No.>3m.Populus | | | 0.145 | | 0.105 | 0.252 |
| Trees.>3m | -0.287 | 0.136 | | 0.106 | -0.158 | |
| Coniferous forest >3m | -0.343 | | | | -0.140 | -0.186 |
| Area | -0.330 | 0.172 | | | 0.107 | |
| Height.m. | -0.307 | 0.162 | | | | |
| Vol | -0.213 | | | | 0.222 | |
| H100 | -0.316 | | | | | |
| Productivity | -0.299 | | | | | |
| Land.productivity | | 0.269 | 0.124 | | -0.341 | |
| Ground.water | -0.195 | | 0.132 | | | 0.258 |
| Ditch.disturbance | | 0.175 | | 0.125 | -0.152 | 0.175 |
| swamps | | 0.189 | | | -0.257 | -0.143 |
| Soil.type | | 0.147 | | | -0.361 | |
| Soil.texture | | -0.132 | -0.226 | 0.119 | -0.182 | |
| Soil.depth | | 0.175 | 0.190 | | -0.126 | 0.105 |
| No.sur.Boulder | | -0.190 | -0.247 | 0.171 | | |
| Boulder.spread | -0.184 | -0.217 | -0.123 | 0.148 | | 0.207 |

| | | | | | |
|---|---|---|---|---|---|
| Boulder.diameter | | -0.176 | -0.228 | | 0.133 |
| Solid.ground | -0.213 | -0.265 | -0.129 | | |
| No.epi.Lichens | -0.208 | | | 0.202 | -0.134 |
| No.dead.stumps | | -0.204 | -0.167 | | 0.188 |
| No.dead.logs | -0.154 | | | | |