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Abstract

Our knowledge on the impact of forest fires on the prevalence (proportion of infected individuals in a population) and dynamics of zoonotic pathogens is largely limited. A large forest fire in late 2006 at Bodträskfors in northern Sweden provided a unique opportunity to investigate the effect of habitat change on disease prevalence. Pummala virus (PUUV) is one of the most prominent zoonotic viruses in this northern boreal forests with bank vole as its only competent host. Human's infection occurs by breathing the aerosolized viral particles shed through saliva, urine and feces of the infected host. The infection causes Nephropathia Epidemia, a milder form of hemorrhagic fever with renal syndrome. The disease has relatively low death rates but can cause lifelong symptoms in humans. Here I have investigated the prevalence of PUUV in bank voles between spring and autumn of 2007-2010 and again in 2015. Small mammals were trapped in trapping plots in the Bodträskfors forest fire area (n=7), mature reference forests (n=7) and unburned clear-cuts (3).

In total 1048 small mammals were trapped from which 1013 bank voles were autopsied and analyzed for anti PUUV antibody with indirect Enzyme Linked Immunosorbent Assay. I used generalized linear mixed effect model to compare PUUV prevalence in the three areas. All bank voles were also weighed and probable weight's correlation with PUUV prevalence was investigated using nominal logistic fit and univariate ANOVA (analysis of variance).

Species composition was one of the most striking results of this study. In the burned area, there appeared to be a one species system, comprised of bank voles only, between 2008-2010 and again in 2015. My results suggest a staggering 78 and 73 percent infection prevalence in burned forest in 2007 and 2015 compared to respective 55 and 44 percent infection prevalence in mature forest. This significant difference was reversed in 2010 with the reference area having the highest infection prevalence (65 to 33 percent respectively). The low species diversity, along with habitat loss due to direct effect of forest fire are suggested to be the two major contributing factors that have led to the very high infection prevalence in forest fire area.

The weight of bank voles was directly correlated with infection prevalence. The weight was highest in spring, in all locations. The burned area consistently had the highest weight average in spring with the reference sites and clear-cut following it respectively. In autumn however, the weight varied slightly between areas without any consistency.

PUUV prevalence differed between the burned and mature reference forests. To pinpoint the exact environmental factors that have resulted in this variation requires further environmental studies, which were out of the scope of this study. The one species system in the forest fire area along with the described infection prevalence portray a unique opportunity for identifying the environment's effect on infection prevalence and also the epidemiologic base of infection prevalence in bank voles with regards to species diversity.

Keywords: Bank vole, Puumala Virus Prevalence, Forest fire, Habitat Loss

Introduction

The bank vole (*Myodes glareolus*) is a small rodent (family of Circetidae), with a wide Palearctic distribution which extends beyond the Arctic Circle in the north and in south, up to northern parts of Turkey and Kazakhstan (Shenbrot and Krasnov 2005). Bank voles infected by Pummala Hantavirus (PUUV) are the zoonotic agent of Hemorrhagic Fever with Renal Syndrome (HFRS). Nephropatia Epidemia (NE) is a milder form of HFRS caused by PUUV. NE has low mortality rate of about 0.4% (Hjertqvist *et al.* 2010) but it can impose long-term effects such as hematuria (with long term consequences), hypertension and proteinuria which can be attributed to the acute kidney injury caused in the acute phase of disease (Latus *et al.* 2015). Host rodents' infection prevalence, defined as the proportion of infected individuals in a population, and the abiotic environmental properties are the determinant factors of viral load in the environment which are suggested to directly influence human infection risk (Miles 2005, Reusken & Heyman 2013). The growing list of countries affected by Hantavirus and consequent increase in human infection rate has made this disease a public health concern (Kruger *et al.* 2013). Better understanding of host infection prevalence as a function of environmental factors could play a significant role in minimizing human infection risks and consequently infection rates by better enabling us to understand and control infection in host population and also better predicting regional outbreaks.

Bank-vole Ecology

Communities of voles and lemmings display a large scale seasonal and multiannual fluctuation in abundance (Korpimaki *et al.* 2004). Long term study of these population cycles by Hörnfeldt (1994, 2004) suggest the cycles to be of three to five-year interval. The magnitude of these population variation is suggested to be up to 500-fold difference between the low and high peak seasons (Korpimaki *et al.* 2004). Multitude of studies have investigated this population cycles. Food scarcity, predation by specialists, disease and weather pattern are only some of the suggested reasons behind these population cycles (Huitu *et al.* 2003, Korpimäk et al 2004, Soveri *et al.* 2000, Haukisalmi and Henttonen 1990).

Bank vole's breeding season is between late April to September (Glass *et al.*1988). A litter can be up to 10 pups but it usually averages between four to eight pups. Females reach sexual maturity after six weeks and males become mature in eight weeks (Lundrigan *et al.* 2003). Larger litter size would result in lower weight and vice versa (Mappes *et al.* 1995). Females maintain territories and their home range is between 500 -2000 m^2 (Haupt *et al.* 2010).

Bank voles reside in a wide range of habitats. However, preferred habitat is dense vegetation, forests and woodland's edges (Viro and Niethammer 1982). Habitat selection is strongly influenced by the direct or indirect habitat composition and structure that provides food and shelter (Ecke *et al*. 2002). Bank voles avoid open areas that would expose them to predators, as so, underground tunnels and undergrowth paths are most often preferred (Lundrigan *et al.* 2003). In peak years however, they can also reach high densities in clear-cuts (Ecke et al. 2002). Despite intrinsic habitat preference, the realized habitat niche can vary in presence of factors such as predators, high density or existence of other species (Lundell *et al.* 2012). The bank vole as the dominant small mammal species of Swedish boreal forests is of crucial importance to the boreal ecosystem by constituting, amongst

other species, as the staple food for many mammalian and avian predators (Hörnfeldt *et al.* 1990).

Hantavirus

Hantaviruses are Bunyviruses and have a genome constituted of three negative sense single-strand RNA segments (Plyusnin and Vapalahti 1996). The 9th report of International Committee on Taxonomy of Viruses (ICTV) identifies 23 established and 30 provisional species in the Hantavirus genus (King *et al.* 2011). Remarkably 51 rodent species are associated with these 51 species of Hantavirus in a "one hantavirus-one host" system (Plyusnin & Sironen 2014). Hantaviruses are a global health concern. This is in part due to their ubiquity, but more so due to their potential in causing sever forms of infection with long-term consequences (Schmaljohn and Hjelle 1997). Globally, up to 150,000 diagnosed cases of Hantavirus infection are reported each year (Johnsson *et al.* 2010). Hantavirus infection in host animal is through two major pathways, indirectly from the environment by inhaling shed viral particles present in feces, urine or saliva of infected rodents, and directly by interaction with infected individuals (Hardestam *et al*. 2008). Pummala virus (PUUV) is a species of Hantavirus genus. This negative-sensed, single-strand RNA virus is associated with bank vole as the only competent (species with high infection transmission capacity) primary host.

Bank vole-PUUV system

Hantavirus's transmission is horizontal and occurs through direct interaction or from inhaling viral particles from the environment. The infection rate can differ as a result of age and sex-specific behavior (Mills *et al.* 1999). Contaminated aerosol is the main route for disease transmission and as such, environmental characteristics can define survival rate of the virus and consequent viral load of the environment (Vapalahti *et al.* 2010). Favorable environmental conditions can result in survival of the virus for days or even weeks (Vaheri *et al.* 2012). Low winter temperature (Olsson *et al*. 2009), moist soil (Linnard *et al.* 2007), low-level UV radiation and humidity are microclimate conditions that favor and thus increase the efficiency of indirect transmission by increasing virus's survival capacity (Guiver *et al.* 2011). Bank voles infected by PUUV disperse virus through their oropharyngeal secretion and feces 14 to 130 days after virus inoculation (Hardestam *et al.* 2008). However, the virus can be absent in some seropositive animals between 191 and 225 days post subcutaneous injection of active PUUV (inoculation) (Yangihara *et al.* 1985). IgG antibody response becomes detectable 18 days after inoculation, peaks after 4-5 weeks and declines again but persists at moderate levels (Yangihara *et al.* 1985).

Adult rodents are generally more susceptible to infection than younger individuals. Maternal antibody present in younger individuals postpones infection and improves individual's breeding success (Kallio *et al.*2006). Individuals become susceptible to infection in about three months as sub-adults (Kallio *et al.*2006). This increase in infection rate of individuals older than three months is further magnified by male dispersal and consequent higher interaction with potentially infected individuals and aggressive behavior (Dolby *et al*. 2012). Host population density and demographic heterogeneity also have significant effect on transmission efficiency and can increase susceptibility to disease (Mills 2005 & Clay *et al.* 2009).

Environmental driving factors of PUUV prevalence

The role of habitat and landscape for disease risk is vastly complex. A comprehensive literature study by Khalil *et al.* (2014) found that in 27 out of 30 studies a positive correlation has been detected between habitat and disease prevalence. The specific relationship between the host and virus makes host ecology the deciding factor in the geographic distribution of the virus (Denis and Dearing, 2010).

It is proposed that landscape composition factors such as forest cover, fragmentation and barrow space, influence the dispersal of voles and consequently the epidemiology of PUUV (Jonsson *et.al* 2010, Salvador *et al.* 2011, Barrios *et al.* 2012). Field studies in Finland by Voutilainen *et al.* (2012) suggest that highest abundance of PUUV infected bank voles are found in forests older than 100 years but the infection rates are highest in young forests aging between 25-30 years. This is despite the fact that over-winter survival of the bank vole is poor in younger forests (Ecke *et al.* 2002 & Savola *et al.* 2013).

Disease prevalence can also be affected directly through environmental condition's effect on survival of the virus in the environment (Voutilainen *et al.* 2012). A study by Linard *et al.* (2007) suggests that low winter temperature is in direct correlation with increased disease prevalence in host, while soil moisture is directly correlated with the number of HFRS cases. A remarkably large outbreak of PUUV in Sweden in 2007 was also associated with peak density season and also reduced snow cover (Olsson *et al.* 2007 & 2009). All in all, habitats structure and quality can dictate the host survival, movement and contact rates, and moreover the survival of the virus and accordingly the viral load in environment.

Biodiversity loss and infection prevalence

All studies on the influence of biodiversity on infection prevalence have consistently found a negative correlation between the two (Khalil *et al.* 2014). The loss of biodiversity is suggested to be the main facilitator of increased infection prevalence in vector-borne zoonosis (Ostfeld & Keesing 2012). Keesing (2001) termed this effect as "dilution effect" predicting that in diverse communities the increase in probability of infection of non-competent hosts will act as a viral sink reducing the probability of competent hosts becoming infected. Biodiversity loss also affects disease transmission by disturbing the abundance, behavior and condition of hosts or vectors (Keesing *et al.* 2006). The review by Johnson & Thieltges (2010) suggests disease transmission to be highly dependent on species composition and diversity. While the term "dilution effect" is generally used to refer to increase in biodiversity of species, Keesing *et al.* (2010) suggest that the diversity of genes, species or even an ecosystem can be expected to influence infection prevalence. This view of the dilution effect is important since it includes the intrinsic capacity of individuals of the same population in avoiding the disease. Anthropogenic factors can be the source of biodiversity loss by inducing local extinction of one or many species, resulting in increased population of more generalist species (for examples bank-vole) and consequently, increased infection prevalence (Khalil *et al.* 2014). The amplitude and magnitude of HFRS outbreaks has increased in the last 20 years in Europe (Reusken & Heyman 2013). The potential of increased or maintained diversity in reduction of zoonotic diseases and promotion of health for humans and wildlife is intriguing but needs to be further investigated.

Forest fire effect on biodiversity and disease prevalence

Forest structure is positively correlated with species richness and abundance of bank voles (Ecke *et al*. 2002). This correlation is suggested to be with tall vegetation and structural heterogeneity of the forest but not with the late successional stages of the forest. Forest fire and logging are two major contributors of compositional and structural change in forest stands (Hart & Chen 2008).

Forest fire can affect population density and composition drastically. A study by Martel (1984), suggests various responses of different small mammals to fire, from rapid decline to rare status in red-backed voles (*Clethrionomys gapperi*), to drastic increase in population size in deer mice (*Peromyscus maniculatus*). While forest fire can be a negative factor for some species, it can also work as a sink for specific species that can cope well with the newly found conditions (Martell 1984). Generalist species such as the bank vole can thrive in such conditions due to lower interference and competition, resulting in an increased population density (Fisher & Wilkinson, 2005). This increase can result in increased disease prevalence and consequently increased human risk of infection (Mills 2006).

A major forest fire occurred in Bodträskfors, Northern Sweden in August 2006 due to a spark by a forestry machine. The fire was the largest forest fire recorded in Sweden until the 2014th wildfire in Västmanland. In total, in 29 days, 1900 ha of productive forest was burned in a total area of 3000 ha (Lundbery *et al.* 2014). This forest fire was severe and burned up to 50 cm of peats and tree roots resulting in some forest patches with 100% tree mortality rate (Johansson *et al.* 2011). In severe forest fires such as that of Bodträskfors, primary and secondary succession occurs (Beyers 2004) in presence of a legacy of species once present at the location and it will take decades for the area to achieve structure and function comparable to its original state (Walker *et al.* 2007).

Aim of Study

The Bodträskfors forest fire provided a unique opportunity to study the effect of forest fire and the consequent effect of change in landscape structure, habitat and small mammal composition on PUUV prevalence. I hypothesized that loss of habitat would initially induce a decrease in population density due to major loss in suitable habitat. However, this was speculated to change to an increase in bank vole density due to the absence of other competing species and plasticity of bank vole as a generalist species. This increase in population, and loss of biodiversity was speculated to cause an increase in PUUV prevalence.

Therefore, this study incorporates two aims. First to analyze the prevalence of PUUV in bank voles between spring of 2007 and autumn of 2010 and spring and autumn of 2015 in the Bodträskfors forest fire area, in comparison to unburned clear-cuts and mature forest located nearby the forest fire. Second, to investigate the possibility of variation in infection prevalence as a result of dilution effect.

Materials and Method

Ethical statement

In order to trap small mammals, permission has been obtained from the Swedish Environmental Protection Agency (reference number 412-4009-10 Nv) and Ethical Committee on Animal Experiments (reference number A39-14).

Study site and Trapping

Small mammals were trapped in the Bodträskfors area located in Norrbotten county (66°N, 20°E), northern Sweden, in spring (early-June) and autumn (mid-September) of each year, from 2007 to 2010 and in spring and autumn of 2015. For the purpose of this study, I have trapped small mammals in 2015; the 2007-2010 trapped small mammals were obtained accordingly. Trapping was performed in permanent trapping plots located in mature forest (n=7), burned area (n=7), and clear-cuts (n=3). Each trapping plot was 1-ha with its position being selected randomly with consideration for avoidance of forest edge, major river systems and roads. Each trapping plot was represented by a 90-m trapping line with 10 stations positioned on an even 10-m distance of the diagonal line of the trapping plot. At each trapping station, five traps were positioned within 1-m radius centered on the trapping station in runways, crevices and covered spaces according to Hörnfeldt & Westerberg (1977). In total 17 plots were trapped using snap traps baited by dried apple and Polish wicks (oil-soaked cotton strings) for three consecutive days resulting in a constant trapping effort of 150 trap-nights per ha-plot. Each captured animal was species-identified to the species level (either in field or in the lab), with time, date and trapping position logged and was given a specific identification code. To avoid cross contamination between captured small mammals, each capture was packed separately and kept in cold condition until transfer to the main holding freezer in which they were kept at -20°C.

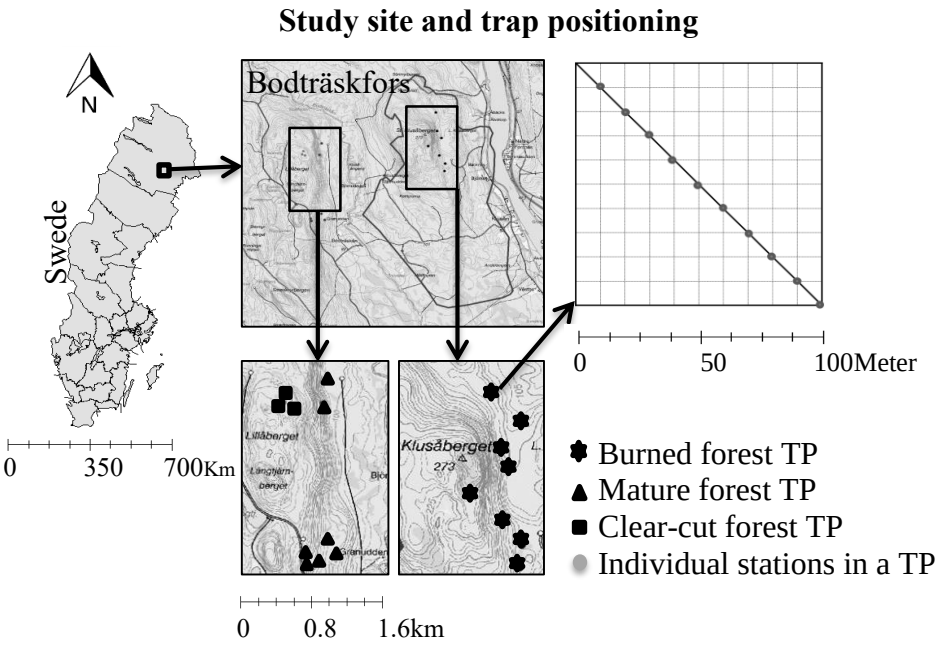

Figure 1: The Bodträskfors area selected for this project. In total seven burned and reference trapping plots and three clear-cut trapping plots were selected, each plot was 1-ha with 10 trapping stations placed along the diagonal of the ha-plot, each having five traps. TP (trapping plot).

Autopsy procedure and Tissue handling
Sample tubes were prepared prior to autopsy procedure by addition of 1 ml of 1% Phosphate Buffered Saline (PBS) solution and five to seven steel ball pestles to each labeled tube. Bank voles were removed from -20°C freezer and when slightly thawed, autopsied using biopsy needle (2*80mm) and 2ml syringe preloaded by 1% PBS solution taken from sample tube. Each rodent was sampled four times; two samples were taken slightly lower than the clavicle bones on either side of the sternum, in a 45-degree angle and two samples at right and left of the approximate location of lower ribs in a 90-degree angle. This process was used to provide autopsy of both upper and lower lobes of the lungs. Samples were then frozen at -20°C. On the day of ELISA test (see below), samples were slightly thawed and fractured twice using Fast Prep shaker with 6.5m/sec frequency for 30 seconds. The resulting sample was centrifuged at 5000rpm (1957*g) for 60 seconds.

Centrifuged samples were investigated using Indirect Enzyme Linked Immunosorbent Assay (ELISA). In this study, Antigen coated plates were prepared in lab. To acquire the best concentrations of Antigen (Ag) and conjugate Antibody (c-Ab), multiple dilution series were run (1 µl Ag or c-Ab in 5, 8,10,15,20,25,30,35 ml). In the first stage, Dilution series of Ag was prepared using Truncated Ag (non-infective) prepared in E-Coli (unknown concentration), and diluted in Coating buffer (0.05 M Bicarbonate buffer, PH: 9.6). Affinity isolated Anti-Mouse IgG alkaline phosphatase Ab (Sigma-Aldrich) was diluted in 1% sterile PBS solution. The titration level, accuracy and precision of the cross dilution series were then investigated. Titration graphs, along with visual observation of consistency in duplicates were used and Ag, Conjugate Ab concentration of 1/30000µl was selected.

Flowingly, 100 µl of diluted Ag was used to coat the ELISA plates. To better disperse coagulated Ag particles present in stock Ag, diluted Ag samples were sonicated with medium power and 50 cycles/min for a total repetition of four times, each consisting of 30 seconds sonication with 15 seconds rest times in between. Plates were antigen coated in every other consecutive row, leaving a blank row for each Ag-coated row.

Controls
Control samples were prepared by pooling high positive samples from previous studies with calculated absorbance higher than one. The pooled sample was then diluted using 1% PBS solution. The resulting diluted sample was then tested for its ELISA based absorbance value and diluted accordingly to read as close as possible to previous positive control's value of one. Low positive control was based on multiple dilution series of high positive control and was run after the cross dilution series.

To determine the lowest detectable level of positive samples, a serial dilution of high positive control was used resulting in a cut-off value of 10 (absorbance of 0.140 at 405nm). This value was used as the cut-off value of positive samples. All samples with absorbance lower than these were considered negative (Crowther 1995).

ELISA test
Samples, control blanks, negative and low positive controls were run in duplicates while high positive control was run in quadruples. 50µl of centrifuged sample was added to Ag coated plates in duplicates and left overnight, the plates were washed four times (200µl

PBS 1 %) and 100µl of diluted conjugate AB was added and samples were incubated for one hour at 37°C. After incubation, plates were washed three times (200µl PBS 1 %), 100 µl substrate solutions (Sigma Aldrich, phosphatase substrate) was added and samples were incubated at 37°C for 30min, after which 5µl one molar NaOH was used as stopping solution and samples were read at 405 nm wavelength using Thermo-scientific plate reader.

To ensure low artifact effect on test results, absorbance adjustment was performed automatically by programing the ELISA reader to use the following formula.

1) $$\frac{(Average\ Sample\ Absorbance - Averager\ blank\ Absorbance\ of\ the\ same\ sample)}{(Averager\ Positive\ Absorbance - Average\ Positive\ Blanks\ Absorbance)} * 100$$

To run the numbers in this formula, the average of Blank duplicates was deducted from all other cells' absorption value. This deduction ensures that increased absorbance due to Ag binding process and possible coloration of plate does not result in false positives. After this step, the formula was run, in which, average of each sample's blank tests (cells without Ag binding) were deducted from the average of the samples test results (cells with bounded Ag). This step ensured that the plate coloration due to high color content of autopsied sample did not produce false positives. Each resulting sample's value was then divided by the mean of the absorbance of known high positive control and the result is multiplied by 100. Since the positive control has a value of one, if the test has perfect conditions, the division would not cause a difference, however if the test has had a subnormal condition resulting in low absorption readout of controls and other samples, the test samples value is divided by a smaller the smaller value of positive control, which would increase the readout of all samples accordingly. Vice versa, a high readout of control would result in adjustment of test samples, by making them smaller. This step ensured that small variations in time and other variants that could affect the test were eliminated and that samples were run in a uniform scale. In case a positive control was misread, all samples were recalculated manually. Since perfect and consistent readout of positive control was required for successful measurement, the entire plate test was performed again if two or more positive samples were misread or read inconsistently.

Removal of Maternal Ab effect

A study by Kallio *et al.* (2010) suggests presence of high degree of maternal antibody in individuals with weights lower than 16 g, while Voutilainen *et al.* (2012) suggest this effect to be prominent in bank voles with weights lower than 14.4g. In this study, 14.4g was used as the threshold, and bank voles weighting less than14.4g were removed from the prevalence studies.

Data Analysis

Species diversity was calculated using the Shannon index to obtain a number that accurately reflects the species composition. The following formula was used in which *Pi* is the relative abundance of species *i* in the community (Whitlock & Schluter, 2009).

2) $$H = \sum(Pi)|ln\ pi|$$

The data on diversity was not further investigated statistically. Since multitude of the results were that of a one species system and no meaningful statistical analysis could be based on them.

Normal distribution of the data was tested using distribution quantile plots and Shapiro-Wilk W test in JMP-Pro (Version 12.1). In spring of all years, weight was normally distributed. In autumn however, the distribution of the data was slightly skewed to the left. This however was present for all sampling groups in autumn. I considered these deviations from normality to be of insignificance value in my statistical analysis. Firstly, according to Box & Andersson, (1995) in large sample sizes, based on central limit theorem, sampling distribution of means behaves correctly for the samples to be tested by parametric tests. Second, according to Whitlock and Schluter (2002) comparing data groups that are similarly skewed to one side should be considered to have normal distribution.

My data passed the four main assumptions of binomial function of generalized linear mixed effect model in SPSS (SPSS Technical report, 2005). First, the dependent variable was measured in a dichotomous scale of zero and one. Second, there was more than one variable (time and weight). Third, the observations were independent and fourth, a linear relationship was present between the variables. The last assumption was tested by SPSS software itself for each set of tests. The SPSS based (V23) generalized linear mixed effect model with binomial distribution, logit link ($f(x) = \log(x / (1-x))$), random effect of habitat and unbounded variance component was used by location and weight for each year. Cox & Snell R square value was used to evaluate the goodness of fit test of the model. The Wald test results were used to determine the statistical significance for each tested variable, and the resulting p values were then used as an indicator of the significance in variation. An SPSS based univariate analysis of variance using SSTYPE 3 method and Post Hoc Tests were used with weight as the dependent variable to identify mean difference from each other in different weight groups in each year and also, in each location. The weight comparison between PUUV positive rodents in each year and location was done using One-way ANOVA analysis of weight as a function of infection and further validated but chi-square test using JMP PRO (Version 12.1).

Results

Small mammals trapped

In total 1048 small mammals of which 1013 bank voles were trapped in the five sampling years of this study (2007-2010, 2015). Of these, 131 (12.7%) small mammals captured, had a weight lower than or equal to 14.4 gr and were considered to hold maternal antibodies which would result in false positive. These samples were removed from the analyses. In general, highest numbers of bank voles were captured in the autumn in all locations and all years. The total number of bank voles captured in autumn constituted 80.3 percent of all bank-voles captured in this study. Highest numbers of bank voles were captured in autumn 2007, 2010 and 2015 with 191, 233 and 247 bank voles, respectively, representing a 4-year cycle of the bank vole, even though no trapping was performed between 2011 and 2014. Total trap-night effort for burned, reference and clear-cut were 1050, 1050 and 450 trap-nights. Clear-cuts had the highest trapping index (number of trapped specimens per 100 trap-nights) in autumn of the peak years of 2007 and 2010 (figure 2 & 3). In both seasons of 2008 and 2009 trappings resulted in very low number of captures. To avoid statistical insignificance, all sampling occasions with sample numbers lower than 10 were removed from the statistical analyses.

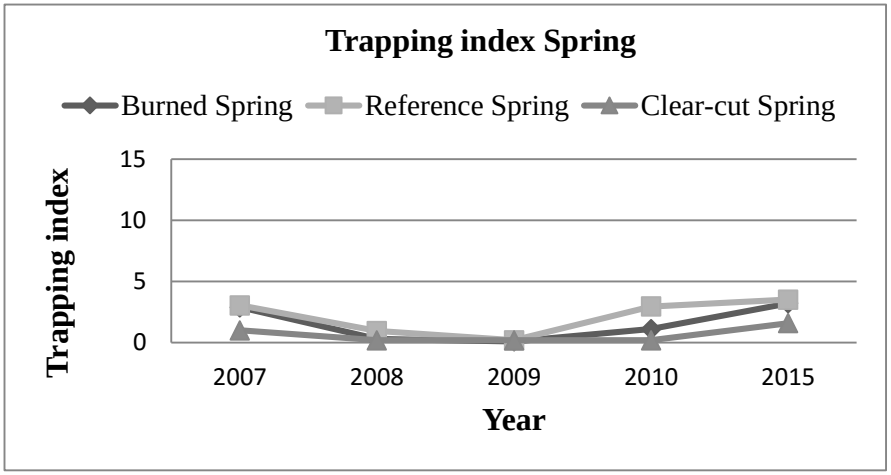

Figure 2: Trapping index measured for 100 trap nights for spring. Highest captures were in peak years (2007, 2010, 2015).

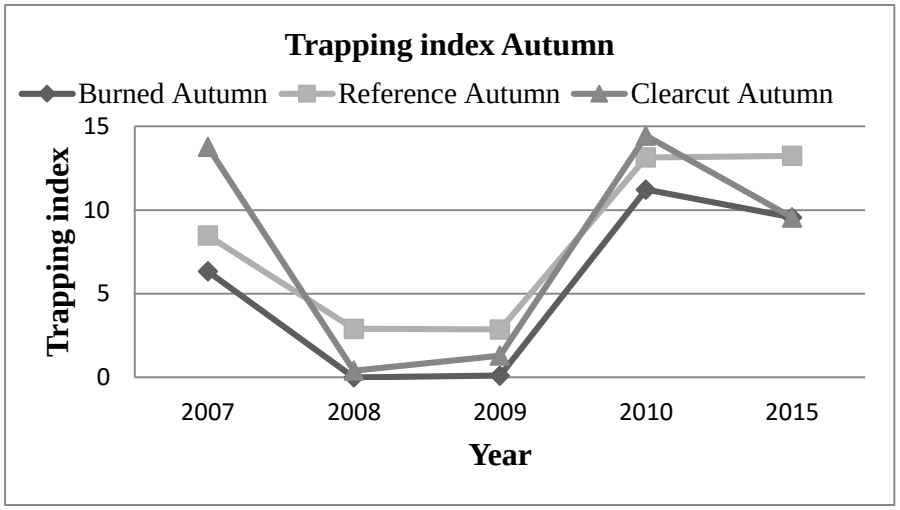

Figure 3: Trapping index for 100 trap nights for Autumn. Similar to spring, highest capture numbers were in peak years in clear-cuts.

Species Diversity and community structure

Species diversity was highest in the burned area at the start of the study (spring, 2007) but became 0.0 in subsequent years (table 1 and figure 4). On the other hand, the reference area showed a relatively low species diversity in 2007 but it increased slightly in the coming years. Diversity in the clear-cut area fluctuated from 0.5 to 0.0 to 0.4. All in all, species diversity was low in the study area with the bank vole as the dominating species present in all years and all seasons.

The bank vole was the most dominant species captured for all years, seasons and locations (99% of all trapped specimens). *Apodemus flavicollis*, *Sorex araneus*, *Myodes rufocanus* and *Sorex araneus* were also captured but comprised less than 1% of the total number of small mammals trapped. One of the main surprising characteristics of the species community structure was the presence of very few individuals of other species than bank vole in the five-year duration of this study. This was especially true in the burned area. In this area, in the last 4 years of sampling only bank voles have been captured (table 1). This is particularly interesting considering that burned forest had the highest species diversity at the start of the study.

Table 1: Species composition of the three areas suggests an abnormally low presence of other rodent species in the fire area for four consecutive years. MG (*Myodes glareolus*), AF (*Apodemus flavicollis*), SA (*Sorex araneus*), MR (*Myodes rufocanus*).

Species Composition by Area & Year (In percentage)

	Burned	Clear-cut	Reference
2007	95 MG/ 2.5 AF/ 2.5 MR	98MG/ 1AF/ 1CR	99MG/ 1MS
2008	100 MG	100 MG	100 MG
2009	100 MG	100 MG	98 MG/ 2 MR
2010	100 MG	100 MG	98 MG/ 1 MS/ 1 SA
2015	100 MG	97 MG/ 3 MR	96 MG/ 4 MR

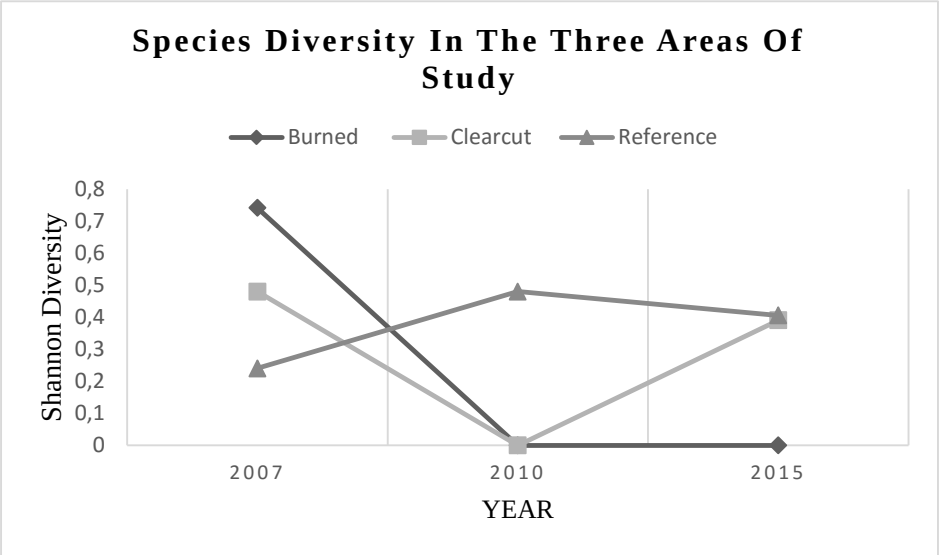

Figure 4: Species diversity index measured for the three years of study with significant capture numbers.

PUUV prevalence

PUUV infection prevalence was exceptionally high in spring of 2007 in the burned area (78%) compared to the reference area (55%). This pattern was reversed in 2010 with reference area having the highest infection rate (65%) compared to that of burned area (33%). In spring 2015 however, the original pattern found in 2007 is repeated with 73 % and 44% for burned area and reference area respectively. In autumn, all samples had considerably lower infection prevalence that was relatively similar amongst all locations. In autumn 2009, the burned area had a higher infection rate (25%) compared to the reference (21%) but reverse was observed in 2010 and 2015 (figure 5).

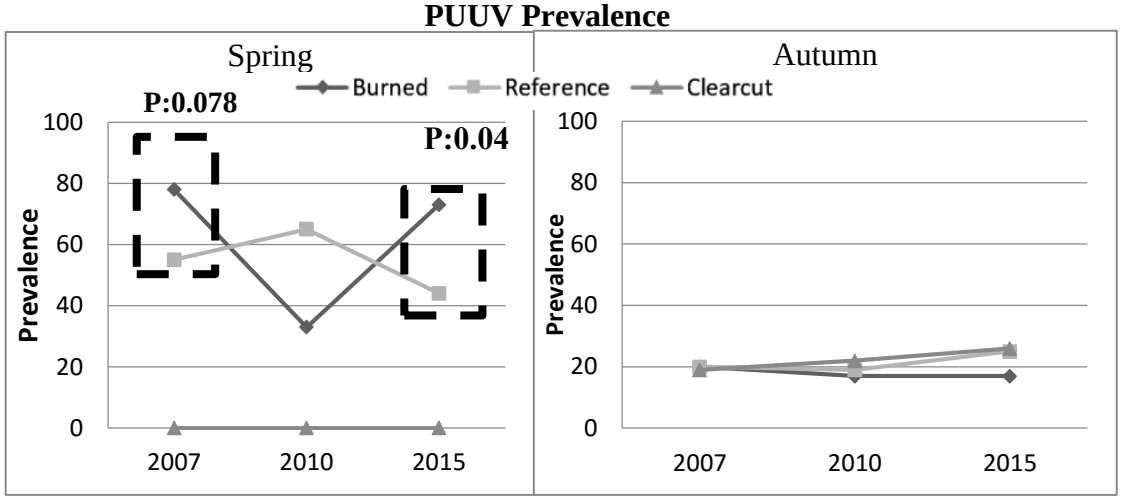

Figure 5: Prevalence of PUUV positive samples per area, location, year and season. Exceptionally high infection prevalence was detected in spring in the burned and reference area. The clear-cut area did not gain enough or any samples for reliable prevalence measurements and is shown as zero in this graph.

Weight correlation with PUUV infection prevalence

Univariate analysis of PUUV variance by weight for all years, seasons and locations (figure 6), supports the proposed positive correlation of weight with infection prevalence. Rate of infection increased drastically as weight increased from 15 to 29-g, from there on however, the infection prevalence remains stable at high levels and even decreased slightly in the last group weighting between 35 and 39 g. The pattern strongly resembles described infection test by Yangihara *et al.* (1985). Seasonal based correlation between bank vole weight and PUUV prevalence suggests low significant correlation in spring and strong correlation in autumn. It is notable that despite the insignificant p value, the slight increase of PUUV prevalence with weight is apparent (figure 7).

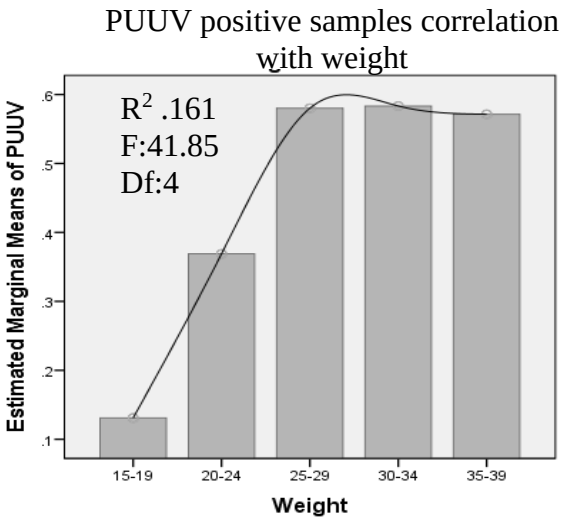

Figure 6: Higher weight can be directly correlated with increased infection rate.

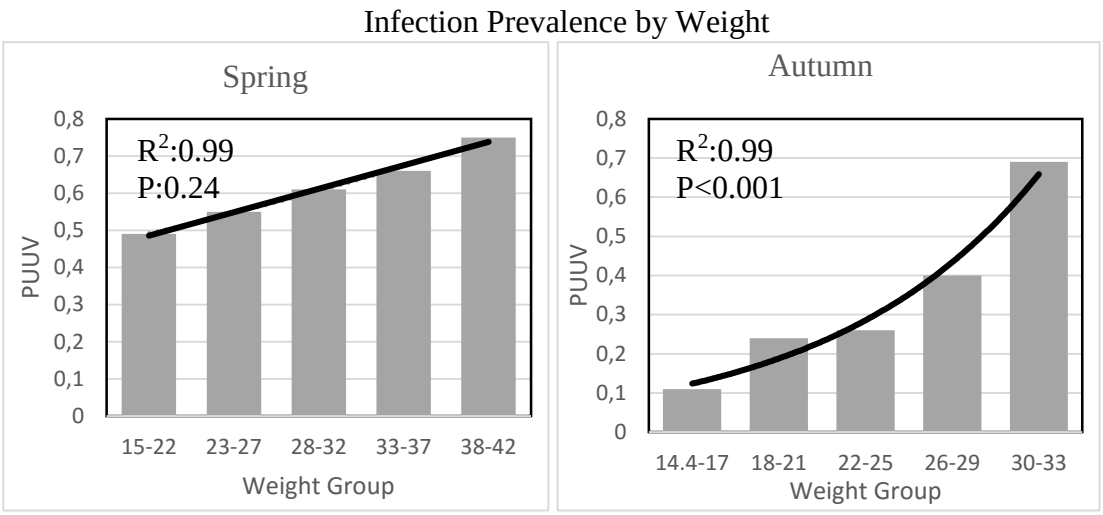

Figure 7: PUUV prevalence by season, measured using nominal logistic fit. Weight was positively correlated with PUUV prevalence in Autumn season.

Figure 8 represents weight as a function of location and year. As expected, spring bank voles were generally heavier than those of autumn. However, in spring, bank voles in the burned area were consistently heavier than bank voles from both reference sites and clear-cuts. One-way ANOVA test of weight between locations suggested high level of difference between weights in different locations in both spring and autumn season. Bank voles in clear-cuts showed constantly lower weight compared to the other two locations; however,

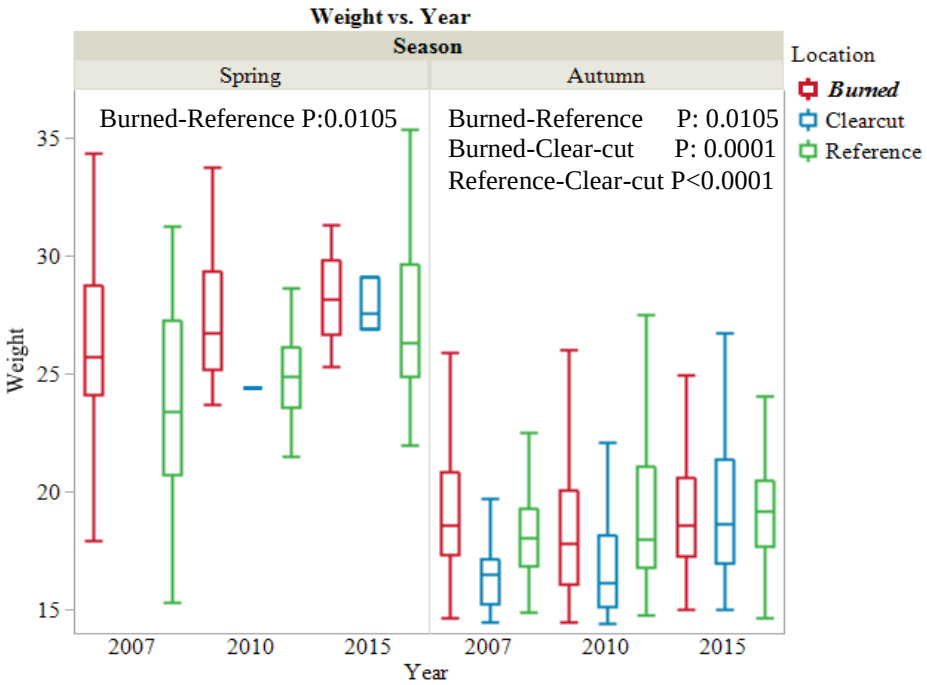

Figure 8: Weight of bank voles as a function of location. Spring season bank voles have a considerably higher weight average than those of autumn.

in spring of 2015 this difference became minimized between the burned area and clear-cuts with both of them containing heavier individuals than the reference area.

Location based difference in PUUV prevalence

Generalized linear mixed effect model in SPSS was used to identify significant differences between PUUV prevalence of the burned and clear-cut area, respectively with the reference area in each year. The result suggested a considerable difference between bank vole infection prevalence in burned forest and reference forest in spring of 2007 and 2015. This difference was minimalized in autumn of all years. And no difference was found in spring of 2010. The same comparison was not possible between clear-cut bank voles and those of reference sites in spring season due to low bank vole number in the clear-cuts. In autumn, the test revealed no significant difference in PUUV prevalence in bank voles when comparing the reference area with the burned area and clear-cuts.

Comparison of infection prevalence in burned and clear-cut with reference area's bank voles without separation of the data by time (year), suggested high differences between burned and reference area's infection prevalence in spring and no difference in autumn. No difference was found between the clear-cut and mature forest.

As expected, weight appeared to have a significant effect on PUUV prevalence both when it was considered for each season of each year separately and also when considered without separation of years. The spring of 2010 and 2015 however appear to be an exception, with no significant effect on infection prevalence.

Table 2: PUUV prevalence in burned and clear-cut area tested against mature forest. Given P values resulted from linear mixed effect model. Individual tests were run for each season of each year and also, without consideration of time constrain (year). Location (randomized) and weight were both considered as model effects.

Linear mixed effect model test evaluation of reference area against burned area and clear-cuts				
P Estimates				
	individual areas compared to reference			Across three areas
Year	**Season**	**Burned**	**Clear-cut**	**Weight**
2007	Spring	0.08	NA	0.003
	Autumn	0.323	0.381	0.0001
2010	Spring	0.29	NA	0.29
	Autumn	0.49	0.1	0.0001
2015	Spring	0.04	NA	0.136
	Autumn	0.12	0.885	0.004
Across study Period	Spring	0.06	NA	0.0001
	Autumn	0.4	0.9	0.0001

Discussions

The large number of small mammals trapped in 2007 with the highest PUUV prevalence coincided with the large outbreak of PUUV infection in humans predicted and reported by Olsson *et al.* (2009). The results from 2007 support the results of Olsson *et al.* (2007, 2009) of expected high infection prevalence in bank voles as a result of high population density of voles in that year. Olsson *et al.* (2009) also suggested warmer winter weather as a major contributing factor to the increased PUUV prevalence in bank voles. Investigation of weather pattern in my trapping locations was out of the scope of this study, however, it can be noted that 2007 has had the highest infection prevalence in comparison to 2010 and 2015 in which high population density of voles were also present.

Clear-cuts showed high trapping indices in the autumn of all years. This seasonal increase in capture of small mammals can probably be attributed to the high population density in adjacent mature forest (core habitat). Habitat selection is strongly influenced by structural habitat factors that provide food and shelter (Hansson 1978, 1997 and Ecke *et al.* 2002). Young forests have a higher heterogeneity and better cover of tall vegetation providing shelter and food for small mammals (Ecke *et al.* 2002). High population density in core habitat along with presence of a heterogeneous and suitable habitat leads to the source sink scenario described by Ecke *et al.* (2002) in which younger individuals of the population primarily born in the old growth forest migrate to the younger forests to breed despite the poor winter survival probabilities associated with this habitat. Similarly, low abundance of individuals in the spring season can be a result of harsher winter conditions, which leads to lower survival rate.

In the burned area, large numbers of small mammals were captured in all years, which might be attributed to the increased heterogeneity of the area as a result of non-uniform fire effect on the forest stands due to increased amount of coarse and fine woody debris. This however, does not explain the low species richness among the small mammal's species found in the last four years of trapping. This drastic decrease could be due to better suitability of bank voles as a generalist small mammal in re-populating and using a changed habitat compared to other small mammals found in the area.

My results are suggestive of presence of exceptionally high PUUV prevalence in bank voles living in the burned area in spring 2007 and 2015 compared to reference area. The apparent similarity of total number of bank voles caught in the burned and reference area suggests presence of factors other than mere population density in PUUV prevalence. It is notable that a study by Voutilainen *et al.* (2012) investigating four-year forest succession pattern, found the prevalence to be 46% in average. Interestingly, their study (Voutilainen *et al.* 2012) also found prevalence as high as 80 percent but only when sampling numbers were very few. In this study, sample numbers lower than 10 were removed from the study resulting in higher statistical power and reliability.

The high infection rate in the burned area can also be attributed to the current and previous patchiness of suitable habitats for bank voles, which could result in aggregation of bank voles, higher contact rate and aggressive behavior. It also needs to be noted that in 2010 the PUUV prevalence decreased drastically in the burned area (from 78% to 33%). This however is most probably a stochastic effect of low number of bank voles captured in spring of 2010 (10 individuals). It can also be speculated that absence of other competing species has enabled the bank-voles to roam freely, this should also be true since the fire area is expected to have considerably lower food source availability and as such bank voles

are expected to travel higher distances increasing the chances of aggressive interactions. In accordance with theory, the lack of incompetent hosts results in higher chance of bank vole infection, supporting the notion of a potential dilution effect on PUUV prevalence (Keesing *et al.* 2009). Better knowledge on vegetation composition, landscape structure and change in microclimate would further our understanding on why and how bank voles have re-populated the burned forest.

In this study weight measurements were used as a determinant factor of age. Weight was directly related to infection rate, this is speculated to be due to the simple fact that heavier individuals are older and as such have had a higher chance of becoming infected in their longer life span (Olsson *et al*. 2002). Weight distribution of bank voles provided further information about the underlying population structure of bank voles. As expected, individuals present in the clear-cut area had the lowest weight compared to that of both burned and reference area. Also the spring population was heavier and older across the study period and locations. Bank voles present in the spring season in the burned area were significantly heavier than that of the reference area. Furthermore, in spring, PUUV-infected bank voles in the burned area had higher weights compared to bank voles in the reference areas. This means probable higher over-winter survival of voles in the burned area or better fire-induced food quality. It can also be speculated that lower top canopy vegetation cover in the burned area had resulted in earlier warming of the forest ground leading to higher winter survival and higher infection based on the same factors that resulted in spring 2007's high PUUV prevalence. Molar tooth investigation of the studied bank voles and providing information on age (Viro 1974) can provide a better answer in this regard.

In autumn, the burned and reference area did not differ in PUUV prevalence across all three years; however, the clear-cut area was highly different with lower PUUV prevalence compared to both burned and reference area. This is expected to be a result of forced movement of younger individuals of the population from core habitat to the clear-cuts, which explains the lower weight associated with the clear-cut area and lower PUUV prevalence associated with the clear-cuts. The total number of samples captured in the autumn is considerably higher than that of spring and as so, the input of high number of bank voles into the population, along with the immunity of newborns against infection by PUUV means that a large proportion of individuals with weights over 14.4 g haven't lived without the maternal antibodies protection for long enough time to become infected. Hence, in autumn, burned, reference and clear-cut areas appear to be similar regarding vole weight and PUUV prevalence.

Understanding the individual environmental factors involved in the mentioned variations was out of the scope of this thesis paper. However, the results of this study portray a unique opportunity, from which, follow-ups and further detailed studies can help to identify environmental effects and dilution effect and also provide further knowledge of the epidemiology of PUUV in bank voles.

Future research,

All in all, this study has resulted in two important results that require immediate follow ups. First, for the first time a one-species system has been identified that provides a unique opportunity for understanding adaptation behavior and dilution effect in a natural system. This system has been identified as a one-species system not immediately after the fire but rather with a two-year delay period. Following this forest system as the succession pattern takes place and other small mammals and vegetation return to this system can provide a unique study of environmental heterogeneity and dilution effect on bank vole population and consequent PUUV prevalence. PUUV prevalence can also be affected by the genetic diversity of the bank vole alongside the environmental factors. In fact, this effect, which is briefly mentioned in Ostfeld *et al.* (2010), has never been studied. If this forest fire has had a reasonable effect on the genetic structure of this population, it could also provide a unique opportunity for studying the dilution effect due to genetic diversity.

Second, this study suggests extremely high PUUV prevalence that needs to be investigated and understood in the epidemiologic aspect of it. The single fact that a forest fire of such magnitude could have triggered such high PUUV prevalence needs to be understood to prevent probable increase in human infection rates. This study confirms previously proven factors such as weight and yearly cycles in regards to PUUV prevalence. It fails however to point out any exact reasons behind the remarkable PUUV prevalence variations that are unique to this study. Environmental conditions of the burned area need to be investigated. It is suggested to evaluate winter temperatures, UV penetration and humidity as the main factors that could affect PUUV survival in the environment.

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