

**Nephropathia Epidemica and Puumala virus
Occurrence in Relation to Bank Vole
(*Clethrionomys glareolus*) Dynamics and
Environmental Factors in Northern Sweden**

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**Doctoral thesis
Swedish University of Agricultural Sciences
Umeå 2003**

Acta Universitatis Agriculturae Sueciae
Silvestria 289

ISSN 1401-6230
ISBN 91-576-6523-0
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Printed by: SLU, Grafiska Enheten, Umeå, Sweden, 2003

Abstract

Olsson, G. E. 2003. Nephropathia epidemica and Puumala virus occurrence in relation to bank vole (*Clethrionomys glareolus*) dynamics and environmental factors in northern Sweden. Doctorate thesis.

The objectives of the thesis were to investigate the spatio-temporal patterns of nephropathia epidemica (NE) in humans and Puumala virus (PUU) occurrence in relation to bank vole (*Clethrionomys glareolus*) dynamics and environmental factors in a region of high incidence of NE in northern Sweden. Nephropathia epidemica is a mild form of hemorrhagic fever with renal syndrome, and in northern Sweden the most prevailing serious febrile viral infection, second to influenza.

All serologically confirmed NE cases during 1991-2001 in the four northernmost counties (n = 2,468) were used to establish spatio-temporal patterns of the occurrence of the human disease. Within the study region, the bank voles show marked population fluctuations with 3-4 yr cycles and the incidence of NE has a temporal component strongly correlated to annual numbers of bank voles in autumn. People living in rural dwellings near coastal areas were abundant among notified cases and middle-aged males were over-represented. The patients were often infected in autumn when engaged in activities such as handling of fire wood, gardening or hay-handling near man-made rodent refugia or cleaning/redecorating within one.

A proportion of these patients, confident about site of PUU exposure, were used to establish field sites in two separate studies. Firstly a five year study (1995-1999) at six sites spanning a bank vole population cycle, and secondly a spatially extensive study at 32 sites was conducted in autumn 1998. Densities, fluctuations and demography of vole populations differ between sites of known occurrence of NE were compared to random forest sites. Five years of repeated biannual sampling revealed that case sites harbored more bank voles than random forest sites, in particular during population peaks. For the individual bank voles, the probability of PUU infection was significantly higher in population peak year, increased with age and was higher for males than for females. In the spatially extended study, it was found that in particular environmental characteristics associated with old-growth moist forests (i.e. *Alectoria* spp., *Picea abies*, fallen wood and *Vaccinium myrtillus*) were associated with high bank vole numbers and numbers of PUU infected bank voles. This implies that success in circulation and persistence of PUU within local bank vole populations is strongly influenced by the local environments.

In future modeling of PUU transmission, influence of bank vole demography and environmental factors should be useful on establishing risk assessments and identifying areas of particular risk of PUU exposure.

Key words: bank vole, *Clethrionomys glareolus*, environmental factors, habitat, hantavirus, hemorrhagic fever with renal syndrome, nephropathia epidemica, population dynamics, Puumala, spatio-temporal.

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Till Carl & Emma

There is no royal road in science, but there is a shortcut through much of this thesis, as illustrated by paraphrasing honorable Frank Zappa:

“Watch out where the bank voles¹ go
and don't you eat that yellow² snow³!”

¹ the natural host to Puumala virus

² indicating common route of virus shedding

³ implying the time of the year of most
nephropathia epidemica cases in Sweden

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Appendix

Papers I-IV

The thesis is based on the following papers, which will be referred to by the corresponding Roman numerals in the text.

- I. Olsson, G.E., Dalerum, F., Hörnfeldt, B., Elgh, F., Palo, T.R., Juto P. and Ahlm, C. Human Hantavirus Infections, Sweden. In press *Emerging Infectious Diseases*
- II. Olsson, G.E., Ahlm, C., Elgh, F., Verlemyr, A.-C., White, N., Juto, P. and Palo, R.T. 2003. Hantavirus antibody occurrence in bank voles (*Clethrionomys glareolus*) during a vole population cycle. *Journal of Wildlife Diseases* 39, 299-305.
- III. Olsson, G.E., White, N., Ahlm, C., Elgh, F., Verlemyr, A.-C., Juto, P. and Palo, R.T. 2002. Demographic factors associated with hantavirus infection in bank voles (*Clethrionomys glareolus*). *Emerging Infectious Diseases* 8, 924-929.
- IV. Olsson, G.E., White, N., Hjältén, J., Palo, R.T. and Ahlm, C. Habitat factors associated with bank voles (*Clethrionomys glareolus*) and concomitant hantavirus in northern Sweden. Manuscript

Papers I-III are reproduced with the kind permission of the publishers.

Introduction

Hantaviruses are the etiological agents, i.e. cause, of hemorrhagic fevers with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). This group of lipid-enveloped and tri-segmented negative stranded RNA-viruses belong to the genus *Hantavirus*, family *Bunyaviridae*, where each distinct form of the virus is associated to one, or possibly a few rodent species. The evolution of the hantaviruses appears restricted to each particular rodent reservoir species within the family *Muridae*, where studies of phylogeny suggest species-specific co-evolution origin millions of years ago (Plyusnin et al. 1996, Schmaljohn & Hjelle 1997). Hantaviruses are maintained in nature by transmission mainly via aerosolized excreta between conspecific rodents, and transmitted to humans commonly by inhalation of hantavirus particles (Xu et al. 1985, Yanagihara et al. 1985, Nuzum et al. 1988, Gavrilovskaya 1990, Bernshtein et al. 1999). Some are seemingly non-pathogen to humans, but others give rise to different forms of clinical manifestations and cause fatality rates ranging from less than 0.5 up to 45% (Linderholm et al. 1991, Valtonen et al. 1995, Young et al. 1998). Several studies have failed to show pathological manifestations or detrimental effects in the natural rodent hosts experimentally infected by hantavirus (Lee et al. 1981, Yanagihara et al. 1984, Childs et al. 1989, Hutchinson et al. 1998).

The hantavirus diseases belong to the emerging infectious diseases that now receive much attention, where the annual number of clinically manifested hantavirus infections in humans on global basis are suggested to be as many as 150,000 (Lee 1996). The number of humans infected from hantaviruses and other rodent borne diseases are assumed to rise as a secondary effect from altered population sizes of rodents in a changing environment due to e.g. global warming changes (Epstein 1995, GCTE Working Document No. 29 1999, Ludwig et al. 2003). For example, the major outbreak of HPS in the southwestern USA in 1993 was associated with locally high abundances of deer mice (*Peromyscus maniculatus*) that are the natural host to the Sin Nombre virus (Childs et al. 1994). The population increase of deer mice followed an abnormally high food production following the El Niño weather event (Chapman & Khabbaz 1994).

The number of identified and official members of the genus *Hantavirus* are at least 22 at present and new species are being identified (Elliot et al. 2000, Meyer & Schmaljohn 2000, Nichol 2001). Only one tenth of the more than 2,000 species within the order Rodentia have been screened yet for their harborage of hantavirus. In 1969, the U.S. Surgeon General William H. Stewart stated before the U.S. Congress that, as result of successful development of vaccines and antibiotics, "it is time to close the book on infectious diseases" (in Ewald 1994). However, evolution of micro-parasites makes no halt and in 1995 the phrase "emerging infections" was coined, i.e. infections that have newly appeared in a population or have existed but are rapidly increasing in incidence or geographic range (Satcher 1995, Morse 1995). Rodents are significant in human health as

reservoirs of zoonotic diseases and emerging infections in addition to those caused by hantaviruses (Mills & Childs 1998).

From an ecological and epidemiological perspective, the population dynamics of the host species is critical to many airborne pathogens for their successful persistence. Dense host populations will facilitate the transmission and local circulation of the pathogen when the transmission occurs directly and horizontally between conspecifics and where there is no bias in the individual's probability of exposure to the pathogen (Anderson & May 1991, Dobson & Hudson 1995, Grenfell & Harwood 1997). Among the rodent species that harbor hantavirus, bank voles (*Clethrionomys glareolus* Schreber) are well known for their temporal changes in population abundance and occasional irruptions in response to e.g. mast seeding (Hansson et al. 2000) for example. Human incidence of hantavirus infection has in general been found to correlate to rodent host numbers especially in the system of nephropathia epidemica (NE; a mild form of HFRS), Puumala virus (PUU) and bank voles (Niklasson & LeDuc 1987, Niklasson et al. 1995, Brummer-Korvenkontio et al. 1999, Escutenaire et al. 2000). In northern Sweden, the only prevailing hantavirus disease is NE, giving rise to an average annual incidence rates of 20-25 serologically confirmed cases per 100,000 inhabitants. As such, NE is the second most common serious febrile viral disease in the region (second to influenza).

The influence of individual characteristics among rodent hosts on the chance of being hantavirus infected has not been fully evaluated. In general epidemiology, age and sex are among the most studied factors on pathogen exposure to individual hosts. Risk of pathogen exposure often increases with age and behavioral differences associated with the sex of the susceptible individual (Anderson & May 1991, Giesecke 1994). Food also plays a critical role in supporting local rodent populations (Ernest et al. 2000, Hansson et al. 2000), and has been suggested to influence occasional outbreaks of hantavirus infections among humans as a consequence of irruptions in rodent populations (Chapman & Khabbaz 1994, Escutenaire et al. 2000). Body condition (\equiv general health) is an important factor correlating with susceptibility to pathogens. Malnutrition could potentially induce immune-suppression among susceptible individuals (Lloyd 1995, Wakelin 1996).

Habitat composition is likely to have an effect on the circulation of hantavirus within local rodent host populations (Verhagen et al. 1986). The effect of habitat composition and local carrying capacity on the persistence of hantaviruses, in long-term and between seasons, has not been fully evaluated. It is, however, likely that sites and phase during population cycle (where cyclic population fluctuations appear) play a critical role on the probability of PUU infection.

Objectives

The objectives of the thesis were to investigate the spatio-temporal patterns of NE in humans, and PUU occurrence in relation to bank vole dynamics and environmental factors in a region of high incidence of NE in northern Sweden. More specifically, the following questions were addressed in the papers I-IV, respectively on which this thesis is based:

- I. Does regional incidence of nephropathia epidemica reveal spatio-temporal patterns related to local bank vole abundances?
- II. Is there a spatio-temporal pattern on the occurrence of Puumala virus among local bank voles in relation to their population dynamics?
- III. What demographic traits influence the probability of an individual bank vole being Puumala virus antibody positive?
- IV. Are there habitat factors that are correlated to local bank vole numbers and/or the presence of Puumala virus?

The study system

Nephropathia epidemica and Puumala virus

The disease NE was first described by two Swedish physicians independently in 1934 (Myrhman 1934, Zetterholm 1934) and the linkage to bank voles was suggested many years later (Lähdevirta 1971, Nyström 1977). Nephropathia epidemica is a milder form of HFRS, where the symptoms range from sub-clinical to renal failure requiring intensive care and dialysis, but fatal cases are rare (Settergren et al. 1989, Linderholm et al. 1991, Mustonen et al. 1994, Brummer-Korvenkontio et al. 1999, Settergren 2000, Linderholm & Elgh 2001). Common symptoms are high fever, headache, nausea, back/abdominal pain, and for about a third of the typical clinical cases also minor hemorrhagic complications and/or renal insufficiency (Settergren et al. 1989). The incubation period of NE is suggested to range between 2 to 5 weeks after infection (Kulagin et al. 1962). Vaccine against PUU infection and specific treatment for NE are still lacking.

Puumala virus is the most widespread hantavirus in Eurasia (Brummer-Korvenkontio et al. 1980, Yanagihara et al. 1984). It was first isolated in 1982 and named after the village Puumala in Finland where the infected bank vole was collected (Brummer-Korvenkontio et al. 1982). Though the virus has been found over most of the bank vole's geographical distribution, some regions appear to be PUU endemic (Verhagen et al. 1986, Bernshtein et al. 1999, Escutenaire et al. 2000), whereas other regions appear to lack the virus.

The incidence of NE is 10-40 reported cases per year per 100,000 inhabitants within the two northernmost counties of Sweden (Settergren et al. 1988, Niklasson et al. 1995). Clinical NE reports, and humans showing PUU antibodies, are rare south of 59° N (Settergren et al. 1988, Ahlm et al. 1998). However, there are southern PUU strains in Sweden carried by bank voles possibly originating from the southern European main continent. A spread from south to the Scandinavian Peninsula was possible during the postglacial period (6,000-8,000 years ago) by using the temporary land bridge between present Sweden and Denmark. There are also northern PUU strains in bank voles of north-eastern origin (Tegelström 1987, Hörling et al. 1996). Nowadays there is a contact zone at around 63° N in Sweden where the two subpopulations meet. The presumed absence of PUU in southern Sweden, as interpreted from the lack of diagnosed NE cases in the region, is an enigma. Yet NE is found as close to the region as the island Fyn in Denmark where up to 22% of sampled bank voles presented PUU infection (Sironen et al. 2002).

The bank vole

The distribution of the bank vole covers most parts of Europe, except some southern regions, and occurs also in Asia. It is probably one of the most numerous small mammal species within its area of distribution (Hansson et al. 2000). The bank vole is found in various types of forested habitats, but also on clear-cuttings and abandoned fields (Henttonen & Hansson 1984) and is both granivorous and folivorous. In northern Sweden they are mostly folivorous, but from late summer through winter they are mainly fungivorous (Hansson 1978, Hansson & Larsson 1978). In northern Fennoscandia the bank vole is commonly most abundant in mature conifer forests (Hansson 1978). Within the northern parts of the bank vole's distribution, the reproductive period begins in early spring and ceases in late September (Nyholm & Meurling 1979). At high population densities, intraspecific social interactions may interrupt the reproduction and maturation of young bank voles (Löfgren 1995).

In Sweden above 60° N, the small mammal community fluctuate synchronously in a fairly regular 3-4-yr periodicity with 100-fold density changes from peak to decline phase (Hansson & Henttonen 1985, Hörnfeldt 1994). These fluctuations are presumed to be food- and/or predator regulated (Korpimäki & Krebs 1996, Krebs 1996). Whatever the ultimate cause of the population fluctuations may be, the variation in bank vole numbers is strongly suggested to be influencing the yearly incidence of human hantavirus disease in northern Fennoscandia (Settergren 1988, Niklasson et al. 1995, Brummer-Korvenkontio et al. 1999).

Methods

Nephropathia epidemica cases

Date and demographic details of notified NE cases within northern Sweden (i.e. the counties of Norrbotten, Västerbotten, Jämtland and Västernorrland) were obtained from laboratory reports from the Departments of Clinical Virology and Clinical Microbiology in Umeå and Boden, Sweden, respectively, covering 1991-1998. The diagnosis of NE was confirmed by the detection of IgM towards PUU with either an immunofluorescence assay or an enzyme-linked immunosorbent assay (Elgh et al. 1996). A questionnaire was sent to the 1991-1998 NE cases concerning activity and location when exposure to PUU was thought to have occurred to obtain data on characteristics of possible exposure sites and risk behaviours. For the remaining part of the period studied, January 1999 to December 2001, numbers and dates of notified NE cases, without demographic details, were obtained from County Medical Officers. The distribution of cases among counties, gender and age classes was compared to the corresponding demographic data for the entire population of the region during the period of our study (Statistics Sweden). The Research Ethics Committee of Umeå University approved those parts of studies that involved the obtaining of data concerning the NE patients.

The study areas and sampling of bank voles

The bank vole sampling studies were carried out in Västerbotten County's coastal areas, in northern Sweden (63°08'-64°45' N; 18°55'-21°00' E). Sampling regimes for voles was performed at sampling sites in the vicinity of a number of NE afflicted human dwellings; 3 sites in the 5-yr study (1995-1999) and 16 in the regional survey, all case sites had paired random sites for comparison.

In the 5-yr study, the NE cases at the sampling sites were serologically confirmed 3-10 weeks prior to first sampling event in autumn of 1995 (case sites South, Centre and North) in the near coastal areas of Västerbotten County in northern Sweden (Ahlm et al. 1997, Paper II). Bank vole sampling was carried out within forest stands of sufficient acreage in close vicinity of the case dwellings (i.e. not within or near man-made rodent refugia). Paired random forest sites to each of the case sites, without known human NE cases, were identified by drawing a 10-km distance radius on a map in relation to the respectively case sites. When sites subsequently were found to be of similar environmental composition as the case site when visited in the field, they were assigned to be used for comparisons on bank vole numbers trapped and occurrence of PUU. The distance of 10 km between paired sampling sites was initially chosen to attain independent sampling within respectively local bank vole population. Voles were

sampled twice a year during 1995 through 1999 in six 300-m long transects covering about 3 hectares, at each site in May-June as soon as possible after the snowmelt and in September-October when bank vole reproduction normally ceased. All sites were similar and composed of managed conifer forests dominated by Scots Pine (*Pinus sylvestris* L.) and Norway Spruce (*Picea abies* L.), with considerable undergrowth of bilberry (*Vaccinium myrtillus* L.) and lingonberry (*V. vitis-idaea* L.).

The larger study was carried out in the autumn of 1998 (bank vole sampling) and summer of 1999 (survey of environmental variables). Within the study region, 101 sites of human PUU exposure were identified, as asserted by NE patients confident about when and where they were exposed to the virus during the period 1991-1996. Of these 101 sites 16 were randomly selected – hereafter denoted case sites – for bank vole trapping. For each case site a corresponding paired control site – hereafter called random site – was used for comparisons on bank vole abundance and habitat composition. A direction from case site to respectively random site was chosen at random between 1-360 degrees. The range of distances between these sites was randomly set to 1-10 km to identify possible spatial correlation between bank vole abundance and PUU. A distance greater than one km was assumed to allow for independent sampling, i.e. greater than the long distance movements described by Gliwicz & Ims (2000). Previous work in the same region (Ahlm et al. 1997) and Paper II, showed that 10 km was far enough to assume independence. Bank vole trapping took place at 32 sites in all (case and random sites). At the center of each case (commonly a dwelling) vs. paired random site, a framework was superimposed with nodes in a 4-by-4 grid, 450x450 m in size. At each node, snap traps set in groups of twelve at up to 16 small quadrates per site (Myllymäki et al. 1971) in autumn 1998. Contrary to the 5 year study the actual case dwellings, or the location found from the map coordinates of the random sites, constituted the center of each sampling site and only if a node within the framework fell on unsuitable matrix, e.g. water, crop or too close to another dwelling, was that node excluded from the sampling regime. Thus, as many as 192 traps were set for three consecutive nights at each site.

Snap-traps were baited with dry apple, checked every morning and local trap success was measured as a trap index (number of bank voles trapped per 100 trap-nights). All sites were sampled within a three-week period, where each pair of case and random forest site was sampled during the same week. Collected animals were kept on ice, transferred to -70° C freezers and later thawed and processed in a bio-safety laboratory (Level 3). See paper I and II for more detail. No pre-baiting was performed in either sampling regime. The sampling technique with snap traps i.e. kill and removal, was permitted by the landowners and the Swedish environmental protection agency (412-3808-01).

Animal processing and serological screening

Body length measurements were obtained to nearest mm excluding the tail, and total body weights were taken to the nearest 0.01 grams (weights of fetus from females were subtracted). Age of 1,079 bank vole individuals, captured in the 5-yr study during 1997-1999, was estimated by criteria from molar root development and growth (Tupikova et al. 1968, Gustafsson et al. 1982).

Blood of bank voles was collected using filter strips (Paper II). Enzyme-linked immunosorbent assay (ELISA) was applied to screen for PUU infection by detection of specific immunoglobulin G (IgG) antibodies in sera (Elgh et al. 1995, 1996). The ELISA-technique in general provides a diagnostic tool specific at the molecular level dependent on the mammalian immunological response. Briefly, the ELISA system uses an antibody that recognizes antigens that are able to bind to the surface of certain plastics (in our studies rodent IgG antibodies towards recombinant PUU antigen). A second antibody, linked to an enzyme, recognizes the first antibody. The enzyme converts a substrate to a colored product, thus giving a possibility to detect the initial antibody by e.g. photo spectrometry (Murray et al. 1998). Each screening by ELISA for bank vole IgG antibodies, included both negative and positive controls, and the cut-off value for being PUU positive was set to 0.2 of the net-absorbance (Elgh et al. 1995, 1996, Ahlm et al. 1997).

Sampling of environmental factors

Within the regional study, 7 pairs of sites were randomly chosen for a survey on the correlation of bank voles and PUU to environmental factors. At each site, all quadrates where bank vole sampling had occurred were included and subject to survey of local environmental factors. Coverage of ground vegetation was estimated within a 0.25m² quadrate at each of the four corners of the small quadrate and at its center. A mean value was calculated for each small quadrate from these five estimates. Tree composition, ground texture and amount of fallen wood, tree stumps and bearded lichens were estimated within a 10 m radius of the small quadrate's center.

Results and discussion

Does regional incidence of nephropathia epidemica reveal spatio-temporal patterns related to local bank vole abundances? (Paper I)

The demographic pattern among identified NE cases within the four northernmost counties of Sweden, showed that middle-aged males were overrepresented (Paper I, Fig. 1). Furthermore, temporal patterns between and within years was found. High NE incidence reoccurred with 3-4 year intervals, a pattern positively correlated to bank vole abundance (Paper I, Fig. 2) and the highest incidence within years was found during autumns and winters. The incidence of NE in autumn throughout the four counties was significantly correlated to the autumn bank vole index recorded in eastern Västerbotten (Paper I, Fig. 3). The incidence of NE in winter was correlated to the bank vole index of previous autumn in Västerbotten only (Paper I, Fig. 4).

From the follow-up questionnaire we also found a finer scaled spatial pattern of the suggested sites of PUU exposure. Replies from NE patients confident about event of PUU exposure, indicated that in particular dwellings in rural and semi-rural parts of the region adjacent to the coast were associated with PUU exposure (Paper I, Fig. 5). These patients claimed that they were infected when engaged in activities such as handling of fire wood (wood piles are very suitable for bank vole nesting), renovating and cleaning dwellings, gardening and hay handling.

In northern Sweden, there seem to have been a geographic shift in occurrence of NE from the inlands towards the coastal areas during the last four decades (Nyström 1977, Paper I, Fig. 5), possibly due to the human de-population of the inlands in favor of the coastal region. The connection between NE and the possible sites of PUU exposure suggested by the patients, strongly indicate the importance of man-made vole refugia (Korpela & Lähdevirta 1978). Awareness of the public to the increased risk of PUU exposure during bank vole population highs and obvious risk activities is essential (i.e. stirring up dust in rodent infested spaces) and likely one of few practical means on how to restrain future NE outbreaks.

Is there a spatio-temporal pattern on the occurrence of Puumala virus among local bank voles in relation to their population dynamics? (Paper II)

In total 1,568 bank voles were captured during 36,840 trapping nights in the 5-yr study, of these 15.4% presented PUU antibodies. Two main results emerged from the study of bank vole on population's level. Firstly, with few exceptions, there were more bank voles captured at the sites with recorded NE as compared to random forest sites (Paper II, Fig. 1), especially during the population peaks (autumns of 1995 and 1998). Secondly, the trap index of PUU IgG antibody positive bank voles increase linearly (density independent) in accordance to the total trap index for autumn populations. However, a density dependent PUU transmission among bank voles would have been expected as high host density are known to facilitate disease transmission (Anderson & May 1991, Dobson & Hudson 1995, Grenfell & Harwood 1997). One plausible explanation for this apparent density independence is dilution due to the relatively large cohort of young bank voles, not yet showing any PUU response. Prevalence, i.e. the proportion of PUU antibody positive bank voles, was normally higher in spring than in autumn, but because the spring catches mainly consisted of few overwintering animals, stochastic outcomes on PUU occurrence in spring cannot be ignored. In the spring of 1998, preceding the second autumn peak during the study, the total trap and PUU antibody positive bank vole indices were significantly higher compared to all other spring samples, irrespective of site (Paper II, Fig. 1).

The intra- and inter-annual fluctuations in the abundance of voles agreed with other studies on the population dynamics of voles in northern Fennoscandia, with the highest abundances in autumns and population peaks 3-4 years apart (Hansson & Henttonen 1985, Hörnfeldt 1994). The incidence of NE within the region co-varied positively with the vole abundance, see also Nyström (1977) and Niklasson et al. (1995). However, the pattern of bank vole numbers and PUU occurrence at sites of previous NE cases that re-emerged during consecutive rodent peaks have not been described previously. The differences in numbers of bank voles trapped on case sites versus random forest sites were very likely due to yet unidentified site-specific features of the local environment facilitating the higher abundances at case sites. Thus, the local presence of PUU virus was not accidental, nor exclusively temporal or consistent. Rather, there was a general temporal component on bank vole numbers following regional vole dynamics. But on a spatial scale there were also differences between sampling sites allowing for site-specific amplitude in population fluctuations (Paper II, Fig. 1). The patterns observed in peak years indicate a re-emerging risk of PUU exposure to conspecifics and man that is habitat and site dependent with a temporal component.

What demographic traits influence the probability of an individual bank vole being Puumala virus antibody positive? (Paper III)

Factors assumed to be associated with PUU infection in 1,000 bank voles in northern Sweden were evaluated by use of binary logistic regression (Paper III, Table 1).

The binary regression predicted >80% of the outcomes correctly, and the significant independent variables were ranked as follows: age; BMI; population phase and sex (Paper III, Table 2). The probability of being PUU antibody positive increased significantly with the age of the bank vole, as expected when considering that opportunities of pathogen exposure in general increase with age (Anderson & May 1991, Giesecke 1994). There are earlier indications that age has an effect on the probability of hantavirus infection to hosts, but these observations are mainly based on rodent weight as a measure of age (Niklasson et al. 1995, Mills et al. 1997, Bernshtein et al. 1999, Escutenaire et al. 2000). I used a more direct age determination method (i.e. molar root development and growth pattern, see Tupikova et al. 1968, Gustafsson et al. 1982) that is advantageous because the body weight of bank voles differs in the course of the population cycle (Hansson 1995) or can be affected by environmental conditions (Hansson 1992).

The body morphology of bank voles, herein measured as BMI, was used in the purpose to distinguish mal-nourished voles from well-nourished ditto. This was done under the assumption that either should mal-nourished voles prove to be more susceptible to infection due to poor body condition (Wakelin 1996) or that bank voles that were well-nourished should reflect lush food abundance at capture sites that attract bank voles (i.e. allowing higher densities) and thus facilitating the PUU spread locally. There was no effect of BMI on the probability of PUU infection; however, a strong relationship with age was observed where juveniles and sub-adults younger than 3 months had a significantly lower BMI than all other groups (Paper III, Fig. 3). This morphological difference is probably associated with sexual maturation and as a consequence, the behavioral changes related to maturation gives rise to more frequent, close contacts with conspecifics and subsequent opportunities of virus transmission. The difference found between the sexes in favor of males on the chance of being PUU antibody positive is likely also due to behavior; males of *Clethrionomys* spp. roam over larger areas than do females and are thus likely to encounter more conspecifics (Ims 1987).

The probability of an individual bank vole being PUU IgG antibody positive was more than twice as high during the vole peak than in the decline phase. I interpreted this as a direct effect due to the density of the bank vole population, as opposed to the delayed density dependence proposed by other researchers (Niklasson et al. 1995, Mills et al. 1999). As the chance of infection also was

lower in the increase phase than the decline phase, the role of having a substantial reservoir of virus infection in the population, i.e. a cohort of infected, overwintering individuals is stressed (Paper III, Fig 1).

Are there habitat factors that are correlated to local bank vole numbers and/or the presence of Puumala virus? (Paper IV)

A total number of 2,173 bank voles were trapped during 15,048 trapping nights in autumn 1998, where 17.6% of these presented PUU antibodies. Among the sites surveyed on habitat features, there were no differences in bank vole numbers or PUU between the paired case- and random sites. Thus, in the subsequent principal component analysis they were evaluated together, independently of original designation.

Increased densities within host populations in many cases facilitate the spread of airborne infectious agents (Anderson & May 1991, Dobson & Hudson 1995, Grenfell & Harwood 1997). However, even if the correlation of bank voles presenting PUU IgG antibodies, to total catch of bank voles was strongly significant, it did not reveal any density dependent pattern. It should be noted, however, that the infected bank vole's humoral response on presenting detectable levels of IgG may lag up to several weeks after infection. Thus, indicating that using a similar analysis on the quicker responding IgM could reveal a direct density dependent pattern, if there is any, where proportionally more bank voles appear PUU infected in denser populations (Murray et al. 1998).

The partial correlation revealed the positive impact of environmental factors assumed to indicate high food availability, i.e. food items such as *Vaccinium myrtillus* (leaves and twigs), *Melampyrum pratense* (seeds) and *Alectoria* spp. lichens (Viro & Sulkava 1985, Hansson 1985, - 1999), together with tree stumps and fallen wood, objects that provide shelter (Tallmon & Mills 1994, Hambäck et al. 2002) and high presence *Picea abies* on the vole trap indices. Bilberry and tree lichens are important food sources for bank voles (Hansson 1985, Viro & Sulkava 1985) and the availability of cover is regarded as a very important determinant for vole habitat selection (Tallmon & Mills 1994, Ecke et al. 2001, - 2002, Hambäck et al 2002). *Pinus sylvestris* on the other hand, is a tree often associated with dry areas with poor coverage of herbs. The results therefore strongly suggest that high abundance of total and PUU positive voles mainly was found in older (indicated by the positive correlation with tree lichens) and moist forest dominated by spruce with good availability of food in the form of bilberry and tree lichens and good availability of cover in the form of logs, tree stumps and bilberry.

The identification of environmental factors related to successful hantavirus circulation and/or persistence within local rodent populations is critical to our understanding of basic hantavirus epidemiology, but also for the implementation and development of precautionary means and increased awareness to the public

on avoiding hantavirus infections. That linking hantavirus circulation to local environmental factors is challenging but rewarding has been described previously (Root et al. 1999, Langlois et al. 2001). The present study provide further knowledge to the understanding of the influence of local environments to the present hantavirus system.

Conclusions and future perspectives

In my thesis I found that: 1) the incidence of NE is strongly correlated with annual numbers of bank voles and reoccurred with 3-4-yr intervals, a pattern positively correlated with bank vole abundance; 2) human activities such as handling of fire wood, gardening or hay-handling near man-made rodent refugia or cleaning/redecorating seemingly increase the risk for PUU exposure to humans; 3) the probability of a bank vole being PUU antibody positive increased significantly with vole's age, and was higher in males; 4) during population cycles the pronounced regional differences in bank vole abundance were revealed and reoccurred at population highs; 5) high vole densities and PUU occurrence mainly is found in older and moist forest dominated by spruce with good availability of food in the form of bilberry and tree lichens and good availability of cover in the form of logs, tree stumps and bilberry bushes.

These findings provide a mean for the further untangling of the ecological web made up of bank voles, PUU and NE. However, many questions remain unanswered, e.g. the transmission of hantavirus within rodent host populations under natural or manipulated conditions are still to a large extent unresolved (Verhagen et al. 1986, Mills et al. 1997, 1999, Abbot et al. 1999, Root et al. 1999, Escutenaire et al. 2000). In particular; there is no clear correlation with rodent host density and proportion of rodents infected (Mills et al. 1999, Hjelle & Yates 2001). Also, significant progress in our understanding of the temporal patterns on PUU transmission among bank voles would be achieved by the use of bank vole IgM response to PUU infection. Presence of PUU IgG antibodies in bank voles mean "infected more than approximately 3 weeks ago" but if no IgG antibodies are found, they may nevertheless be recently infected, or not infected at all. As the IgM response occurs more rapidly its use would be more likely reveal density dependence in Puumala virus transmission between bank voles, if there is any to be found.

Many airborne micro-parasites circulate in a density dependent manner where higher prevalence of the pathogen are found within increasing host populations and where sometimes endemic "hot spots" can be identified (e.g. Grenfell & Harwood 1997). The risk of pathogen exposure often varies as a result of environmental heterogeneity (Anderson & May 1984, Verhagen et al. 1986, Ostfeld & Keesing 2000). The identification of environmental factors likely to cause the patterns observed on PUU occurrence and bank vole dynamics is

essential to the understanding of circulation and persistence of the PUU in bank vole populations. The qualitative evaluation of local microhabitats, potentially serving as micro-refugia during population lows, is also critical (Dobson & Hudson 1995, Grenfell & Harwood 1997).

There are, however, alternative hypothesis to density dependence on PUU circulation and persistence. Firstly, a frequency dependent PUU transmission may explain why similar proportions of the studied bank vole populations presented PUU antibodies, irrespective of number of bank voles captured per sampling area. Thus the number of contacts between infectious and susceptible individuals may remain much the same as a consequence of e.g. constraints on individuals movements because of territoriality and/or an upper bound on number of sexual contacts (Begon et al. 1999, Begon et al. 2002). If our observations hold true that only a minority of young bank voles are Puumala virus infected when also tested for IgM response, then it is likely that Puumala virus transmission is strongly coupled to behavioral mechanisms among adult bank voles, i.e. defending territories and/or fighting for mates. Whether PUU transmission within bank vole populations is density dependent or frequency dependent, or perhaps a combination of the two when populations within restricted areas increase in numbers (Mazurkiewicz 1994), needs to be considered when attempting to model the system. Secondly, Sauvage and others (2003) suggest when modeling the PUU – bank vole system that indirect transmission (long lasting “survival” and infectiousness of PUU outside the host in the “right” environment e.g. humid ground conditions) was one of the most influential factors on PUU persistence within bank vole populations thus uncoupling some of the importance of bank vole population size to the successful transmission of PUU (Sauvage et al. 2003).

To identify which of the processes above, acting alone or in concert, that have the strongest influence on successful PUU persistence and circulation within bank vole populations is of uttermost importance to understand when developing models of PUU transmission. The bank vole – PUU system in Fennoscandia is convenient for its relative simplicity (e.g. few other co-existing and competing rodent species; well-studied population processes; temporarily “predictable” population fluctuations through the regional 3-4 year periodicity, not caused by the PUU) and warrants further studies, including monitoring by capture-mark-recapture studies in well-defined habitats. In combination with laboratory animal models, this would create opportunities to explore individual-based models of PUU transmission and maintenance in host populations. This system may therefore also serve as a model to hantavirus systems in general on these aspects.

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Summary in Swedish

Förekomst av nephropathia epidemica – sorkfeber – och Puumalavirus i relation till skogssork (*Clethrionomys glareolus*) och miljöfaktorer i norra Sverige

Många vanligt förekommande infektionssjukdomar har ett zoonotiskt ursprung, det vill säga att den sjukdomsalstrande organismen/mikroparasiten (ex virus och bakterier) härbärgeras naturligt av, och sprids från andra ryggradsdjur till människor. Eftersom mikroparasiter som regel har en kort generationstid och förmåga till snabb evolutionär anpassning till nya miljöer och andra ändrade förutsättningar, finns det en ständig risk för att nyupptäckta eller gamla zoonotiska infektionssjukdomar kan orsaka stora personliga lidanden och kostnader för samhället, trots motåtgärder i form av exempelvis vacciner och bekämpningsprogram.

Föreliggande doktorsavhandling beskriver och diskuterar förekomsten av infektionssjukdomen nephropathia epidemica – eller sorkfeber – i norra Sverige, i relation till värdjuret skogssork och det hantavirus de sprider. Sorkfeber är, efter influensa, den vanligaste allvarliga virala febersjukdomen i norra Sverige.

Sorkfeber är en zoonos orsakad genom infektion av Puumalavirus, som har skogssork (*Clethrionomys glareolus*) som naturlig värd. Puumalaviruset hör till den växande gruppen *Hantavirus*, familjen *Bunyaviridae*, där respektive virus förknippas med en specifik art bland sorkar, råttor, möss eller lämlar. Till dags dato är cirka 22 olika hantavirus identifierade. Hantavirus utsöndras och sprids från infekterade smågnagare via saliv, urin och avföring. Infektion av hantavirus – både hos gnagare och människor – sker vanligen genom inandning av luftburna viruspartiklar. Tillsammans orsakar dessa virus årligen uppskattningsvis mer än 150,000 människors insjuknande i världen. Andelen människor som dör i sjukdomar orsakade av hantavirusinfektion varierar stort, från mindre än 0,5% (sorkfeber) till cirka 45% (Hantavirus pulmonary syndrome). I ett globalt perspektiv hör norra Sverige till en av de regioner som uppvisar de högsta talen av diagnostiserade fall av hantavirus smitta per invånare – i genomsnitt 25 fall per 100,000 invånare och år – undantagslöst rör det sig här om sorkfeber. Mörkertalet bland smittade i norra Sverige är stort; för varje diagnosticerat fall förblir 7-8 oupptäckta.

Skogssorken är en av Europas vanligast förekommande och mest studerade smågnagare. Förekomsten av Puumalavirus har konstaterats i lokala skogssorkpopulationer i flera delar av kontinenten, från västra och centrala delar av Europa till europeiska delarna av Ryssland i öst. Utmärkande för skogssorkpopulationerna i norra Skandinavien är de tämligen regelbundna 3-4 års cyklerna med mycket stora variationer i sorktillgång mellan topp- och bottenår. Orsaken till

dessa fluktuationer är fortfarande källa till diskussioner, men tillgången på föda respektive rovdjur hör till de faktorer som spelar roll.

Mer specifikt är avhandlingen baserad på följande frågeställningar. Resultat och diskussioner är utvecklade i detalj i de respektive uppsatser som presenteras i avhandlingen (uppsatserna I-IV):

Finns det samband i tid och rum mellan sorkfeber i norra Sverige och förekomst av skogssork? (uppsats I)

Skiljer sig förekomsten av Puumalavirus mellan lokala populationer av skogssorkar? (uppsats II)

Vilka demografiska faktorer hos enskilda sorkar är förknippad med infektion av Puumalavirus? (uppsats III)

Finns det faktorer i miljön som bidrar till ökad lokal förekomst av skogssork och Puumalavirus? (uppsats IV)

Finns det samband i tid och rum mellan sorkfeber i norra Sverige och lokal förekomst av skogssork? (uppsats I)

Sorkfeber är sedan 1989 en anmälningspliktig infektionssjukdom där varje diagnostiserat fall skall rapporteras. Med det rapporterade patientmaterialet som grund gjordes en enkätstudie riktad till sorkfeberpatienter diagnostiserade från 1991 till och med 1998 och med ytterligare fallen fram till 2001 ur smittskyddsregister i de fyra nordligaste länen (totalt 2,468 fall av sorkfeber); Norrbotten; Västerbotten; Västernorrland och Jämtland.

Resultaten visar att det är främst medelålders män som återfinns bland de diagnostiserade fallen (män i åldrarna 25-74 och kvinnor 45-59 är relativt överrepresenterade i patientmaterialet); smitta förknippas ofta med aktiviteter i, eller i anslutning till åretrunt- eller fritidsboende i de kustnära delarna av de fyra nordligaste länen. Det senare resultatet framgår av enkätsvaren där 862 patienter som var säkra på vistelseort vid tillfället för virusexponering fick besvara frågor om detta. Bland dessa patienter ägnade sig 27% åt hantering av ved; 19% städade eller renoverade en byggnad och 18% ägnade sig åt trädgårdsarbete eller hantering av hö. Den övervägande andelen av personer smittas och insjuknar under perioden oktober till och med mars, med de flesta fallen under hösten. Detta sammanfaller i tiden med att virusets naturliga värdar, skogssorkarna, inte längre hävdar revir (som under sommarens reproduktiva period) och att vinterhalvårets klimat kan bidra till att mänskliga boningar och vedhögar blir attraktiva för sorkarna till skydd mot framförallt kyla och väta men också rovdjur. Sambandet mellan tillgången på skogssork under tidiga hösten och antalet insjuknade patienter med sorkfeber under höst och vinter är tydligt. Sjuttiofem procent av variationen i antalet fall av sorkfeber från Västerbotten i studien under perioden oktober-december förklaras med hjälp av den positiva korrelationen till fångsten av skogssorkar i en pågående långtidsstudie i länet. Sambandet är statistiskt

signifikant också för de tre övriga länen i studien – i relation till skogssorkdata från Västerbotten – om än samvariationen inte är lika starkt.

Skiljer sig förekomsten av Puumalavirus mellan lokala populationer av skogssorkar? (uppsats II)

Under 1995 initierades en studie där förekomst av Puumalavirus och populationsdynamik hos skogssorkar studerades i tre områden med nyliga fall av sorkfebersmitta i Västerbottens kustområde, en studie som pågick till och med hösten 1999. Till vardera tre lokalerna fanns en parad slumpad lokal med liknande naturtyp (brukad barr- och blandskog) som jämförelse. Varje höst och vår under perioden 1995-1999 fångades skogssorkar vilka analyserades avseende immunoglobulin G antikroppar mot Puumalavirus.

Resultaten visar att det på lokaler med känd sorkfebersmitta fanns fler skogssorkar än på jämförelselokalerna. Andelen sorkar smittade av Puumalavirus var lika mellan lokaler, men det totala antalet smittade var fler på lokalerna med känd sorkfebersmitta. Detta samband var genomgående för hela studieperioden, men statistiskt signifikant i samband med toppåren 1995 och 1998.

Vilka demografiska faktorer hos enskilda sorkar är förknippad med infektion av Puumalavirus? (uppsats III)

Ettusen åldersbestämda skogssorkar användes för att förstå betydelsen av demografi och andra faktorer i relation till förekomsten av Puumalavirus smitta i enskilda skogssorkar. Sorkarna var insamlade i den ovan beskrivna fältstudien under åren 1997-1999, under vilken ett år med populationstillväxt (1997), ett toppår (1998) och ett år med nedgång i sorkpopulationerna (1999).

I den logistiska regressionsmodell som användes för analys av faktorer, förutsäger fyra av de ingående faktorerna 80% av modellens utfall. De fyra faktorerna är – i fallande ordning av inflytande – skogssorkens ålder; kropps-konstitution ("body mass index" BMI); fas i sorkpopulationernas fluktuationer som sorken fångats (tillväxt/topp/nedgång), samt skogssorkens könstillhörighet.

Konstaterad smitta hos de ingående skogssorkarna var positivt korrelerat med ålder: ju äldre djur desto större andel smittade. Mellan de fyra åldersklasserna som skogssorkarna indelats i, framgår att de som uppnått köns-mognad och sannolikt varit aktiva under sommarens reproduktion i större utsträckning exponerats mot Puumalavirus än de som ännu ej var köns-mogna vid infångandet, respektive de som överlevt vintern men infångats före reproduktionssäsongen. I kategorin "skogssorkar som övervintrat och fångats på hösten av sitt 2:a levnadsår" hade 75% konstaterad smitta av Puumalavirus.

Vid undersökning av skogssorkars kropps-konstitution i relation till smitta av Puumalavirus visade det sig att ju kraftigare sork, desto större var sannolikheten att vara smittad med Puumalavirus. Detta är ett resultat som skall tolkas med viss försiktighet. Den bakomliggande faktorn som troligen indirekt ger detta resultat, kan vara förknippad med skogssorkarnas köns-mognad (och därmed ökade aktivitet) då i synnerhet hanarnas kropps-konstitution förändras. Den fas under skogssorkarnas populations-fluktuationer då sannolikheten är störst att skogssork är infekterade av Puumalavirus är under toppåren. Mellan skogssorkar av hon-respektive hankön, är sannolikheten att vara smittad av Puumalavirus störst bland hanar. Detta kan vara en effekt av dessas större rörlighet i samband med den reproduktiva perioden, och därmed fler (aggressiva) kontakter med andra skogssorkar.

Finns det faktorer i miljön som bidrar till ökad lokal förekomst av skogssork och Puumalavirus? (uppsats IV)

Ett antal lokaler med säkra tidigare fall av sorkfeber, och kontrollområden till dessa, studerades med avseende på förekomst av skogssork, Puumalavirus och sammansättning av lokala miljöfaktorer. Resultaten visar att det finns miljöfaktorer som i studien är signifikant förknippade med större antal skogssork och därmed förekomsten av Puumalavirus. Bland de faktorer som utmärker sig finns sådana som direkt eller indirekt förknippas med föda och/eller skydd för skogssorkarna. Till dessa hör som föda blåbär (*Vaccinium myrtillus*; löv, bär och kvistar), ängs- och skogskovall (*Melampyrum pratense*, *M. sylvaticum*; frön) och olika hänglavar (*Alectoria* spp.). Dessa utgör födoslag för skogssorkar i regionen. Stubbar och kullfallna träd hör till de faktorer som i analysen är signifikant positivt förknippade med antalet fångade skogssorkar, och de utgör skydd för skogssorkar, så även blåbärsris. Förekomsten av gran (*Picea abies*) var positivt relaterad till antalet skogssorkar, medan förekomsten av tall (*Pinus sylvestris*) tvärtom var negativt korrelerad till skogssork. De båda trädslagen representerar olika slags miljöer, där gran ofta förknippas med fuktigare och mer näringsrika miljöer, medan tall förknippas med torrare och näringsfattigare dito.

Analyserna ger stöd för uppfattningen att fler skogssorkar, och därmed förekomsten av Puumalavirus, kan relateras till äldre och fuktiga skogar (indikerat av förekomsten av hänglavar) som med betydande inslag av gran, med god tillgång på föda i form av blåbär och hänglav, samt skydd i form av stubbar, fallna träd och blåbärsris.

Att identifiera miljövariabler som kan relateras till förekomsten av Puumalavirus bland skogssorkar är av stor betydelse för att förstå den grundläggande epidemiologin i systemet. Denna kunskap är också av vikt för att kunna utveckla modeller för virus-spridning inom skogssorkpopulationer i olika

miljöer och för att öka kunskapen om potentiella riskmiljöer för människor att exponeras för Puumalavirus.

Sammanfattande slutsatser

I norra Sverige insjuknar flest människor i sorkfeber under vinterhalvåret, och då i synnerhet under oktober-december. Många blir smittade av Puumalavirus i eller i anslutning till åretrunt- eller fritidshus, och ofta i vedbodas, uthus eller liknande utrymmen. Sorkfeberpatienter i studien förknippade ofta smittotillfället med aktiviteter då damm rörts upp och andats in med vidhängande utsöndrade, infektiösa Puumalavirus partiklar.

Lokaler i fält med säkra fall av sorkfeber har identifierats med hjälp av uppgifterna från ett antal patienter använts. På dessa lokaler utfördes två olika studier med syfte att identifiera lokala skillnader i skogssorkars populationsdynamik respektive lokala miljövariationers inverkan på förekomsten av skogssorkar och Puumalavirus. Resultaten från de sex lokalerna i 5-års studien visade att det vid dessa lokaler med känd sorkfebersmitta råder en vidmakthållen större sannolikhet för såväl skogssorkar som människor att exponeras för Puumalavirus. Studien som omfattade ett fångstillfälle men även insamlandet av miljövariabler visar på betydelsen av lokala miljöbetingelser avseende förekomsten av skogssork och Puumalavirus. I ett större perspektiv ger det skäl att anta att det finns en samvariation mellan risk för exponering av Puumalavirus och lokala miljöbetingelser, med en koppling i tid till sorkpopulationernas fluktuationer.

Långlivade, övervintrande skogssorkar – om än fåtaliga i populationen som helhet – framstår som betydelsefulla för Puumalavirusets vidmakthållande på lokal skala. De är då Puumalaviruset lokalt ej återfanns bland insamlade skogssorkar, var under populations fasens bottenår då inte heller övervintrande skogssorkar återfanns lokalt. Puumalavirus överföring sker framförallt mellan köns mogna skogssorkar under sommarhalvåret, och då i synnerhet mellan hanar.

Acknowledgments

I owe a debt of gratitude to all the people involved – mentioned and unmentioned – whose contribution to my life and work made this thesis come true.

Firstly, I would like to thank my supervisors over the years, through whom I have gained insight in many fields and topics. Clas Ahlm whose ideas originally made the study become real and my enrolment to the project possible. Thank you, Clas! I am grateful for learning so many rewarding and interesting things from and through R. Thomas Palo, useful not only within the academics. I am glad that Joakim Hjältén always had time to read and discuss various topics and quite often managed to put my wild thoughts back on track. Neil White entered the project at a late stage but contributed most significantly to the outcome of the project and my thesis. Professor Kjell Danell and Kjell Sjöberg founded the creative environment where I have spent most of my time awake (and occasionally asleep) over the past years.

Professor Per Juto was among the initiators of the nephropathia epidemica-project in Umeå and was together with Ann-Christin “Anci” Verlemyr no less than the backbones to the project. Professors Göran Wadell and Arne Tärnvik provided for the facilities to make the studies and analysis possible. Fredrik Elgh, Oleg Alexeyev and Mats Linderholm, ”products” of the earlier era of the project, provided fruitful discussions on the topics of virus, voles and mammal fitness.

I am most grateful to all the people making peak performance in the field and laboratories: Fredrik Dahl; Fredrik Dalerum; Ulla Eriksson; Johan Green; Anna Henningson; Mikael Kärnlund; Åsa Laurell; Patrik Nordin; Åke Nordström; Roslyn Schumacher; Per Wedholm; Hans Werthén and Johan Örnerkrans.

Anders Hansson and Peter Mortensen at the Swedish Museum of Natural History were invaluable in the handling of more than a thousand of bank vole carcasses preceding the age determination of these voles. These aged specimens (the voles!) are today incorporated into the collections of the museum.

Birger Hörnfeldts immense data set on small mammal trappings is a cornucopia in this study system I am very pleased to have had the opportunity to use.

I thank all the near innumerable, and here anonymous, nephropathia epidemica patients that so willingly have participated in this study, and I am grateful to the landowners that allowed for the sampling of small mammals on their land.

Friends and colleagues at the Department of Animal Ecology, Swedish University of Agricultural Sciences, and at the Division of Infectious Diseases respectively Division of Virology, Department of Clinical Microbiology, Umeå

University, provided interesting discussions in a challenging environment, and challenging discussions in interesting environments.

Professor Terry L. Yates who made it possible for me to spend invaluable time at his hantavirus research project in New Mexico, U.S.A., moreover where he and Professors Bob Parmenter and Jim Gosz with workers and Field Station Manager John Dewitt made my stay at the University of New Mexico and Sevilleta Field Station an unforgettable one.

Most part of this work was financially supported by grants from the Centre for Environmental Research (CMF 952114), the Swedish Medical Research Council (K-2000-06X-10382-08A), the County Council of Västerbotten and the European Community (contract no. BMH4-CT97-2499). The Research Ethics Committee of Umeå University approved the part of the study involving humans. Trapping was permitted by landowners and the Swedish environmental protection agency (412-3808-01).

Tack!

Ni är oräkneliga, alla Ni som på ett eller annat sätt bidragit till den här avhandlingens tillblivelse och de krumbukter som varit på vägen hit!

Clas Ahlm, R. Thomas Palo, Joakim Hjältén och Neil White har fungerat som mina bollplank, mentorer och som handledare under åren som gått. Lösa bollar, hårda bollar, men framförallt många bollar har det blivit! Tack för god match med hård träning och karaktärsdanande coaching!

Sorkfeberprojektet initierades av bland andra Professor Per Juto som tillsammans med Ann-Christin "Anci" Verlemyr spelat avgörande roller i projektets framgångar. Bland tidigare doktorander finns Fredrik Elgh, Oleg Alexeyev, Mats Linderholm och Clas Ahlm förstås, som bidragit till att göra projektet till den spännande smältdegel av discipliner och kulturer som präglar tvärvetenskapliga arbeten.

Stort Tack! till kolleger och vänner som tillsammans skapar kreativa miljöer med spännande diskussioner och spännande miljöer med kreativa diskussioner vid de tre institutioner och avdelningar jag varit knuten till under de här åren, med Professor Kjell Danell och Kjell Sjöberg i spetsen för alla skogliga zoökologer (SLU); Professor Göran Wadell och alla vid viruslaboratoriet (Umeå universitet) samt Professor Arne Tärnvik och medarbetare på infektionskliniken (Umeå universitet).

Min avhandling hade aldrig blivit vad den är utan alla sega medarbetare i fält och laboratorier: Fredrik Dahl; Fredrik Dalerum; Ulla Eriksson; Johan Green;

Anna Henningson; Mikael Kärlund; Åsa Laurell; Patrik Nordin; Åke Nordström; Roslyn Schumacher; Per Wedholm; Hans Werthén och Johan Örnerkrans. Ert anletes svett (blod? tårar?) och tålamod ligger bakom varje siffra i avhandlingen.

Anders Hansson och Peter Mortensen på Naturhistoriska Riksmuséet bidrog med ovärderlig hjälp att preparera insamlade skogssorkar av vilka 1,000 så småningom blev åldersbestämda och inkorporerade i muséets samlingar.

Ett extra stort Tack! till alla sorkfeberpatienter – här av nöden anonyma – som genom enkätsvar och intervjuer gjort mitt arbete alls möjligt, och alla markägare som upplåtit sin mark åt oss att samla sorkar och andra data!

Genom Tomas Willebrands förtjänst hamnade jag på ekologins brokiga bana ("When the going gets tough..." har en annan klang idag, eller hur? ☺) och när jag arbetade åt Professor Vidar Mareström försvann all tvekan om att vägvalet var absolut det rätta.

I Södra Tuna, Dalarna, hos familjerna Ingman och Arvidsson, har jag en oas som jag bara behöver tänka på för att pigga till. Hoppas få se Er mycket mer framöver!

Några har ofrivilligt fått stå tillbaka, mer eller mindre, under åren som gått: Carl och Emma – mina två solar på himlen – era leenden är min största belöning; Anna, mina barns mor; min egen mor och mina syskon.

Ett oändligt Tack till alla som inte får plats att nämnas; tro mig Ni är inte glömda!!

och Inger, som får mig tro att änglar finns...