National Institute
for Public Health
and the Environment

Letter report 330361001/2009
C.B.E.M. Reusken

Hantavirus infections in the Netherlands, a risk profile

rivm

Hantavirus infections in the Netherlands, a risk profile.

RIVM Letterreport 330361001/2009.

C.B.E.M. Reusken,

Contact:
C. Reusken
Centrum voor Infectieziektenbestrijding,
Laboratorium voor zoönosen en omgevingsmicrobiologie
Chantal.Reusken@RIVM.nl

Dit onderzoek is uitgevoerd door Cib-LZO van het RIVM in opdracht en ten laste van de Voedsel en Waren Autoriteit in het kader van project V/330361/01/HA.

Contents

Summary and Recommendations.

In most European countries the incidence of hantavirus infections has increased over the last couple of years. An extension of the hantavirus endemic areas has been observed for Belgium, France, Germany and the Netherlands, including an invasion of urban regions in Germany. Experts predict that with the anticipated climate changes, disease caused by hantaviruses might become highly endemic in northern and western Europe.
These observations were reason for the Dutch Food and Product Safety Authority (VWA) to ask for a risk profile concerning the public health risks of hantaviruses in the Netherlands as a first step towards a risk assessment. This risk profile is limited to hantaviruses circulating in Europe and focuses in particular on Puumala hantavirus (PUUV) and its rodent host *Myodes glareolus* ([Rosse woelmuis]).

The factors that influence or regulate human risks at hantavirus transmission are numerous and complex, and cover a variety of disciplines including human and animal virology, ecology, immunology, zoology, geography and mathematics. Multidisciplinary research and monitoring is a prerequisite for a proper assessment of the risks for public health and to initiate timely and effective intervention strategies.

In summary, the following remarks and recommendations can be made based on an extensive search into literature addressing risk factors for human hantavirus infection:

Human diagnostics.
1) In general the discriminatory ability of the diagnostic tests used in the Netherlands is considered as sufficient since only PUUV is expected to circulate. This risk profile shows that human infections with Tula virus (TULV) can't be excluded while infection with Seoul virus (SEOV) should seriously be considered. Therefore a more discriminating diagnostic setup should be implemented in case further typing of the infecting hantavirus type is required. Besides improving our diagnostics, a more discriminating setup would improve the necessary knowledge about the infecting hantavirus types and their epidemiology in the Netherlands.

2) Reports on the circulation of Dobrova virus (DOBV) in *Apodemus flavicollis* ([grote bosmuis]) in surrounding countries should be monitored closely to assess the possibililty of DOBV circulation in *Apodemus flavicolis* populations in the Netherlands. In case DOBV infections are considered as a serious option for the Netherlands, more discriminating diagnostics will be required as DOBV and SEOV belong to the same serogroup with known high crossreactivity but with differences in the severity of illness .

Recommendation.
To improve the human hantavirus diagnostics in the Netherlands. The establishment of more discriminating human hantavirus diagnostics in the Netherlands will enable the exact typing of infecting hantavirus types and related pathogenicity and improve our knowledge on hantavirus epidemiology in the Netherlands.

Recommendation.
To closely monitor the international developments in hantavirus circulation in rodent reservoirs and human outbreaks, with a focus on the advancement of new types of hantaviruses from adjacent countries, in particular DOBV and SEOV.

Epidemiology.
3). The factors that influence and regulate the human risks for hantavirus infections are numerous (figure 10). The PUUV (and hantaviruses in general) transmission risk for humans depends on a combination of:

a) host ecology,
b) virus ecology
c) human behavior.

a) Host ecology involves environmental factors related to bank vole population densities and structure, *viz*. land-surface attributes, landscape configuration and climate. Climatic factors control vegetation growth, snow cover and food supply, f.e. mast production.
b) Virus ecology involves environmental factors (both microclimatic and chemical parameters) related to virus survival outside the rodent host, *viz*. winter temperature, level UV-radiation, soil pH and soil moisture. Virus ecology influences PUUV prevalence in a bank vole population and human transmission risk through the indirect transmission route.
c) Some human activities will be associated with a close contact with the host habitats and thus increase the likelihood of human-host contacts or with a close contact to areas that support virus survival.

The spatial variation in human PUUV infections is predominantly determined by virus ecology in combination with human activities while the temporal variation in human PUUV infection risks is predominantly determined by the abundance of infected bank voles at a given time, which is influenced by both virus and host ecology (figure 10).

4) For a proper assessment of the risks for human hantavirus infections in the Netherlands it is necessary to determine the current hantavirus seroprevalence in the Dutch population and the temporal-spatial variations therein.

Recommendation.
To determine the current hantavirus seroprevalence in the dutch population and the temporal-spatial variations therein. This could be determined using the PIENTER sera present at the CIb/RIVM[1].

Geographic distribution in the Netherlands.
5). Based on the presence of their specific rodent hosts, four different types of hantaviruses might circulate or emerge in the Netherlands:
a) PUUV through the reservoir host *Myodes glareolus,*
b) TULV through the reservoir host *Microtus arvalis* ([veldmuis]),

[1] For 2009 a screening of a cohort of the PIENER sera is planned. The criteria for the cohort have not been defined yet.

c) SEOV through the reservoir hosts *Rattus rattus* ([zwarte rat]) and *Rattus norvegicus* ([bruine rat]),
d) DOBV through the reservoir host *Apodemus flavicollis*.

6). The circulation of PUUV and TULV in respectively *Myodes glareolus* and *Microtus arvalis* has been confirmed for only a few locations in the Netherlands. The current data on the spatial distribution of hantavirus circulation in the Netherlands is too limited for risk assessment purposes. Furthermore, research into hantavirus circulation in the dutch rodent populations should be extended beyond these two species and should include at least other *Microtus spp*. and *Rattus sp*. in relation to SEOV circulation and if indicated by new developments amongst others *Apodemus flavicollis*.

7). It is not justified to assume that hantaviruses only circulate in areas of human disease. The currently available data for the Netherlands do not exclude the presence of hantaviruses in broader areas than the currently recognized endemic zones based on human cases.

8) The potential risk for public health of hantavirus emergence is likely to be greater than believed based on the distribution of human cases. Disturbances in stable demographic patterns in rodent populations can lead to the emergence of known and unknown hantaviruses in the human population in areas considered free of hantaviruses.
Therefore, for risk assessment purposes, as a measure for the human transmission risk that could incur following sudden changes in bank vole demography, it is important to improve our knowledge on the general distribution of hantavirus circulation in rodent hosts in the Netherlands.

9) Spatial and temporal variations in infection rates in any given rodent population can vary from high to even absent. For risk assessment purposes endemic areas should be monitored to follow trends in time.

10) Based on the high rate of forest fragmentation in the Netherlands, a very patchy/local presence of PUUV in areas were bank voles are present can be expected even if the local conditions are optimal for PUUV circulation.

Recommendation.
To expand our knowledgde on hantavirus circulation in putative reservoir hosts in the Netherlands for risk assessment and disease prevention purposes.
As a measure for the human transmission risk that could incur following sudden changes in rodent population structures and densities, it is important to assess the general distribution of hantavirus circulation in rodents in the Netherlands. Endemic areas should be monitored to follow trends in time to get more insight in the dynamics of rodent-borne hantavirus infections in the Netherlands. Field studies should not be limited to *M. glareolus* and *M. arvalis* , but should include *Rattus* sp., *Apodemus* sp. and other *Microtus sp*.

Considering the expected patchy/focal presence of PUUV in areas with bank voles in the Netherlands, an option for a cost-effective and “quick-and-dirty” route to map the *current* human risk areas for PUUV infections in the Netherlands would be the

assessment of the hantavirus seroprevalence in the Dutch population and the temporal-spatial variations therein. However this will not provide information on the risks for emergence of known and unknown hantaviruses in areas considered free of hantaviruses based on human cases nor will it, with the current human diagnostics, provide the necessary insight in circulating hantavirus types and rodent species involved.

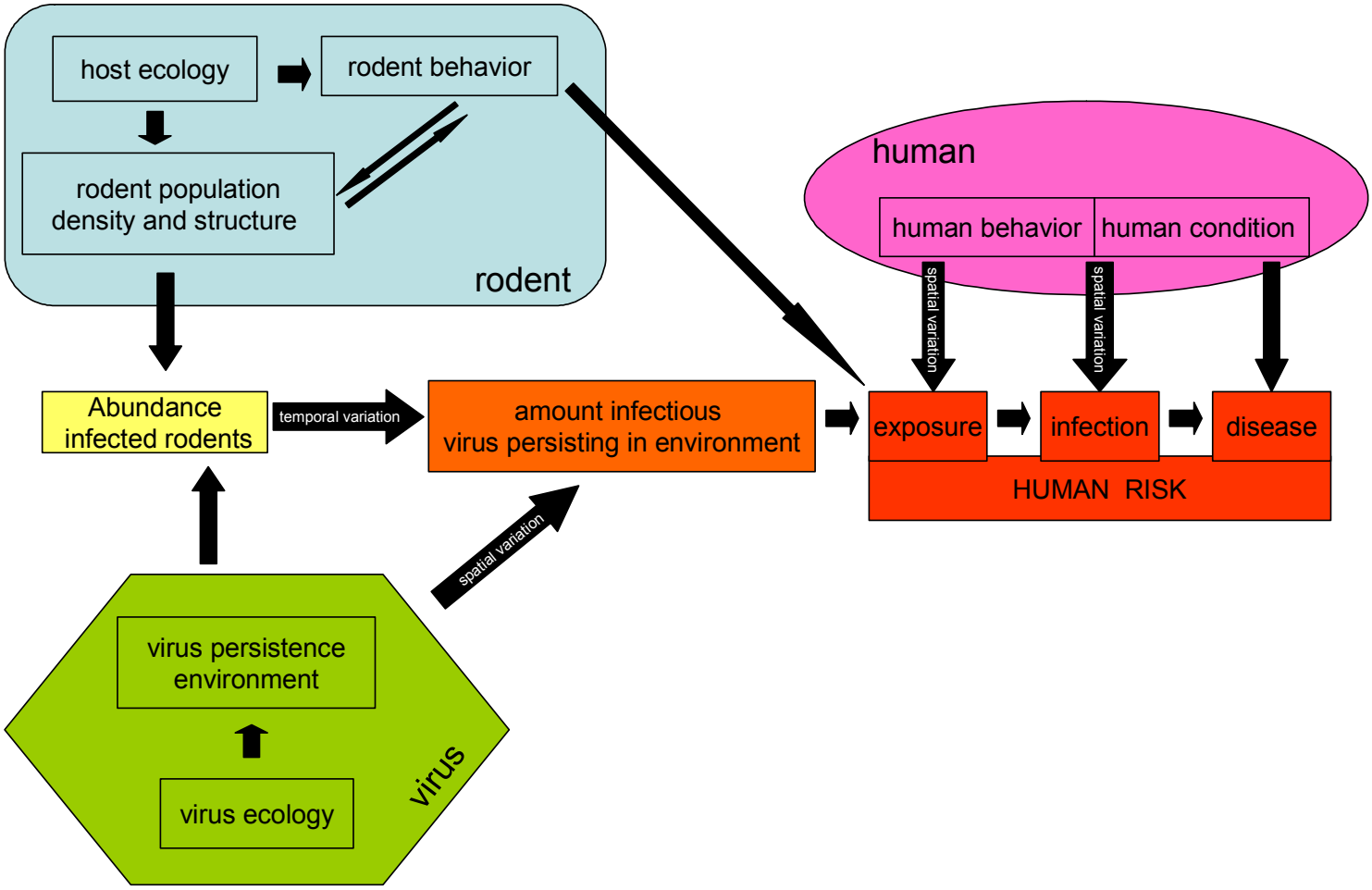

Figure. Flow chart factors influencing the human risk for hantavirus infection.

Early-warning.
11). Above a threshold abundance of bank voles, virus ecology will better predict the nephropathia epidemica (NE) incidence in humans than host ecology, in particular the degree of soil moisture and the maximal temperatures from the preceding winter. This implies that beyond this threshold, an increase in the population size does not necessarily lead to an immediate increase in risk for human transmission.

12) For Belgium it was observed that homogeneous high seed production of both beech and oak is closely related to an increased incidence of NE in Belgium. Based on this observation and the known correlation between both bank vole abundance/high seed production and bank vole abundance/human PUUV infection risk, it can be concluded that when beech, oak or both show high seed production in autumn, this can be regarded as an early warning tool for public health policy makers.

13) For Belgium it was observed that elevated average summer temperatures followed by warm autumn conditions in the next year constitute a direct link to increased human PUUV infection risk in the year following this autumn. This in combination with the mast years mentioned at 12) can be considered as early warning indicators for NE outbreaks in Belgium. This can be extrapolated to neighbouring countries, including the Netherlands. Experts believe that the NE mechanics will be fundamentally the same in these countries as they are in the same biome and have a similar documented presence of the bank vole.

Recommendation.
To set-up a systematic early-warning system for the timely detection of public health risks due to the abundant presence of hantavirus infected rodents. Indicators for high rodent densities and virus circulation therein, involving parameters concerning host ecology and virus ecology, should be identified and continuously monitored.

Most importantly, as the bank vole and other rodents/insectivores are known reservoir hosts for other emerging pathogens in the Netherlands and Europe, including *Toxoplasma spp., Babesia spp., Giardia spp. Cryptosporidium spp. Mycobacterium spp. Borrelia spp., Echinococcus multilocularis*, tick-borne encephalitis virus, Ljungan virus, Hepatitis E virus and cowpoxvirus, the predictive factors for hantavirus risks should be integrated preferably in a general rodent monitoring programme.

Recommendation.
To set-up a general systematic early-warning system from a broader perspective including the risks of high rodent densities for public health in general, animal health, biodiversity and agricultural damage. The early-warning system for hantavirus risks should preferably be integrated in such a general rodent monitoring system.
This monitoring system for rodents could be used to predict and facilitate recommendations for persons/animals in at-risk areas for a wide variety of diseases.
This monitoring system needs to be set up as a collaborative effort of Wageningen University and Research, Faunafonds, Zoogdiervereniging VZZ, CVI, Alterra and CIb/RIVM.

Control measures.
Control measures are based on:
1) avoiding contact with rodents, their excreta and aerosolized virus particles,
2) decontamination.
Rodent control in nature will interfere deeply with excisting local ecosystems which is undesirable and illegal in the Netherlands based on the “Flora en Fauna wet”

Recommendation.
To draw up an information brochure about the risks of hantaviruses and the control measures that can be taken.

Recommendation.
To arise public awareness by dispersal of information brochures to populations at risk f.e. through general practitioners, camp grounds, leisure centers and tourist information offices in known hantavirus endemic regions and through the relevant health and safety executives.

With the recommendations for research, and monitoring given above priority areas and high risk periods for public health policies, aimed at preventing disease by informing the public and promoting the use of protective measures, can be defined and redefined.

1. Background.

In most European countries, the incidence of hantavirus infections has increased over the last couple of years [1 2 3 4 5]. An extension of the hantavirus endemic areas has been observed for Belgium, France and Germany, including an invasion of urban regions in Germany [6]. Experts predict that with the anticipated climate changes, disease caused by hantaviruses might become highly endemic in northern and western Europe [7,8].
These observations were reason for the Dutch Food and Product Safety Authority (VWA) to ask for a risk profile concerning the public health risks of hantaviruses in the Netherlands as a first step towards a risk assessment.
This risk profile is limited to hantaviruses circulating in Europe and focuses in particular on Puumala hantavirus (PUUV) and its rodent host *Myodes glareolus* (bank vole, [Rosse woelmuis]). In northern-western Europe, PUUV is the most commonly circulating hantavirus causing the majority of human hantavirus cases.

2. Hazard identification.

2.1. Etiology pathogen.

Hantaviruses belong to the genus hantavirus of the family of the *Bunyaviridae*. The family of the Bunyaviridae comprises of 5 genera, 4 of which contain representatives that are zoonotic (tabel 1). Nairoviruses, phleboviruses and bunyaviruses are arthropod-borne viruses (transmitted by arthropods like ticks, sandflies and mosquitoes), hantaviruses are rodent-borne (transmitted by rodents).

Tabel 1. Family *Bunyaviridae*.

genus	**Representative(s)**	**Human disease**
Nairovirus	Crimean-congo hemorraghic fever virus	Crimean-congo hemorraghic fever
Phlebovirus	Rift Valley fever virus	Rift Valley fever
Bunyavirus	La Crosse encephalitis virus	La Crosse encephalitis
Hantavirus	Sin nombre virus Puumala virus	Hantavirus pulmonary syndrome Hemorraghic fever with renal syndrom
Tospovirus	Tomato spotted wilt virus	-

Bunyaviruses have enveloped virions varying 80-120 nm in diameter, containing a segmented negative-stranded RNA genome. The tripartite genome is approximately 12 kb long and encodes four proteins. The small (S), medium (M) and large (L) genome segments encode respectively the nucleocapsid (N), two glycoproteins (G1,G2) and the RNA-dependent-RNA replicase (RdRp) (figure 1).

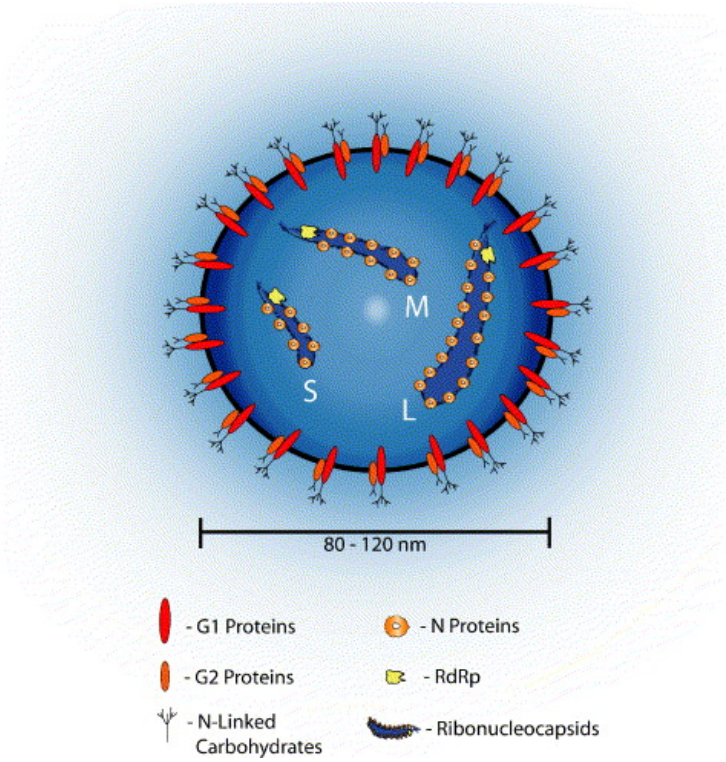

Figure 1. Schematic representation of a bunyavirus.

The genus hantavirus is subdivided into three groups based on the taxonomic classification into subfamilies of their rodent carriers (see 2.2), *viz*. *Arvicolinae*-associated (voles, lemmings) , *Murinae*-associated (Old World mice, rats) and *Sigmodontinae*-associated (New World mice, rats) viruses. Genetically closely related rodents carry genetically closely related hantaviruses. Phylogenetic studies show that viruses associated with the rodent subfamilies *Arvicolinae*, *Murinae* and *Sigmodontinae,* form each a distinct phylogenetic branch. The Old World rodent-associated viruses are genetically more closely related (Figure 2). Nearly identical phylogenetic trees can be constructed when rodent host mitochondrial genes and viral gene sequences are analysed [9]. A study of the molecular evolution of PUUV strains in northern Europe showed that the genetic variation within a hantavirus type is related to the geographic distance of their rodent hosts which depends on the ancestral rodent migration routes [10]. These data suggest that hantaviruses have co-evolved with their rodent hosts for million of years [11].
Currently, over 40 different serotypes have been identified of which 23 are known human pathogens (Table 2).

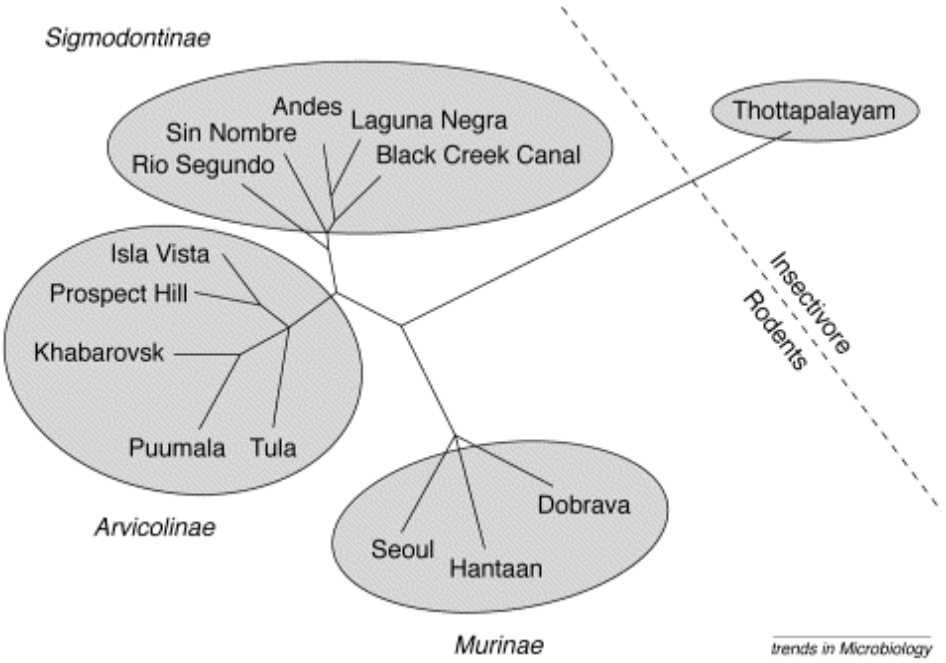

Figure 2. Phylogenetic relationships of hantaviruses based on alignment of amino acid sequences of the nucleocapsid protein (N). The subfamilies of hantaviral rodent hosts (*Murinae*, *Arvicolinae* and *Sigmodontinae*) and the insectivore host are designated by the shaded regions. Accession numbers for S segment sequences (from which the amino acid sequences for N were derived) are: Andes virus, AF004660; Black Creek Canal virus, L39949; Dobrava virus, L41916; Hantaan virus, M14626; Isla Vista virus, U19302; Khabarovsk virus, U35255; Laguna Negra virus, AFOO5727; Prospect Hill virus, X55128; Puumala virus, X61035; Rio Segundo virus, U18100; Seoul virus, M34881; Sin Nombre virus, L33683; Tula virus, Z48235. *Thottapalayam* S segment (pers. Comm.) Amino acid sequences were aligned using the Clustal W Multiple Sequence Alignment Program Version 1.7. Phylogenic relationships were displayed by using TreeView tree drawing software. (taken from [12]).

2.2. Vector

2.2.1. Primary reservoir hosts.

Hantaviruses are maintained by cyclical transmission between persistently infected rodents (primary host), with incidental infection of other mammalian hosts including humans (so called spillover or accidental host). Each hantavirus type appears to have co-evolved with a specific primary rodent species [9]. Currently 22 distinct hantaspecies are officially recognized by the International Committee on Taxonomy of Viruses. In total > 40 isolates are recognized. An increasing number of hantaviruses has been described that are associated with insectivores [13-19]. Their association with human disease has not been established. Table 2 gives an overview of the to-date recognized hantaviruses and their primary rodent carriers. Primary rodent carriers become persistently infected with their associated hantavirus type, and shed virus in saliva, urine and feces. They have a crucial role in the epidemiology of hantaviruses.

Virus	Primary rodent host (order Rodentia; family Murinae)	Location first detected	Human disease	Reference
Arvicolinae-associated				
Puumala *	*Myodes glareolus*	Finland	Mild HFRS/NE	[20]
Tula *	*Microtus arvalis/M. rossiaemeridionalis*	Russia	Mild HFRS	[21]
Prospect hill *	*Microtus pennsylvanicus*	Maryland, USA	None recognized	[22]
Bloodland lake	*Microtus ochrogaster*	Missouri, USA	None recognized	[23]
Isla Vista *	*Microtus californicus*	California, USA	None recognized	[24]
Khabarovsk *	*Microtus fortis*	Russia	None recognized	[25]
Topografov *	*Lemmus sibericus*	Siberia	None recognized	[26]
Hokkaido	*Myodes rufocanus*	Japan	None recognized	[27]
Muju	*Myodes regulus*	Korea	None recognized	[28]
Murinae-associated				
Dobrava * (DOBV-Af)	*Apodemus flavicollis*	Slovenia	Severe HFRS	[29]
Saarema (DOBV-Aa)	*Apodemus agrarius* (western form)	Finland	Mild HFRS	[30]
Seoul *	*Rattus norvegicus, Rattus rattus*	Korea	Moderate HFRS	[31]
Hantaan *	*Apodemus agrarius* (eastern form)	Korea	Severe HFRS	[32]
Sangassou	*Hylomyscus simus*	Guinea	None recognized	[33]
Soochong	*Apodemus peninsulae*	Korea	None recognized	[34]
Thailand *	*Bandicota indicus*	Thailand	None recognized	[35]
Amur	*Apodemus peninsulae*	Russia	HFRS	[36]
Sigmodontinae-associated				
Sin Nombre *	*Peromyscus maniculatus*	New Mexico, USA	HPS	[37]
New York *	*Peromyscus leucopus*	New York, USA	HPS	[38]
Black Greek Canal *	*Sigmodon hispidus*	Florida, USA	HPS	[39]
Bayou *	*Oryzomys palustris*	Louisiana, USA	HPS	[40]
Muleshoe *	*Sigmodon hispidus*	Texas, USA	HPS	[41]
Monongahela	*Peromyscus maniculatus*	Pennsylvania, USA	HPS	[42]
Limestone Canyon	*Peromyscus boylii*	Arizona, USA	None recognized	[43]
Blue river	*Peromyscus leucopus*	Indiana, USA	None recognized	[44]
El Moro Canyon *	*Reithrodontomys megalotis*	New Mexico, USA	None recogniezed	[45]
Rio Segundo *	*Reithrodontomys mexicanus*	Costa Rica	None recognized	[46]
Caño Delgadito *	*Sigmodon alstoni*	Venezuela	None recognized	[47]
Juquitiba	*Oligoryzomys nigripes*	Brazil	HPS	[48]
Araraquara	*Bolomys lasiurus*	Brazil	HPS	[48]
Castelo dos Sonhos	*unknown*	Brazil	HPS	[48]

Virus	Primary rodent host (order Rodentia; family Murinae)	Location first detected	Human disease	Reference
Rio Mamoré *	*Oligoryzomys microtis*	Bolivia	HPS	[49]
Laguna Negra *	*Calomys laucha*	Paraquay	HPS	[50]
Andes *	*Oligoryzomys longicaudatus*	Argentina	HPS	[51]
Lechiguanas	*Oligoryzomys flavesces*	Argentina	HPS	[52]
Bermejo	*Oligoryzomys chacoensis*	Argentina	HPS	[52]
Orán	*Oligoryzomys longicaudatus*	Argentina	HPS	[53]
Hu39694	*unknown*	Argentina	HPS	[53]
Maciel	*Bolomys obscurus*	Argentina	None recognized	[53]
Pergamino	*Akodon azarae*	Argentina	None recognized	[53]
Choclo	*Oligoryzomys fulvescens*	Panama	HPS	[54]
Calabazop	*Zygodontomys brevicauda*	Panama	None recognized	[54]
Maporal	*Oecomys bicolor*	Venezuela	None recognized	[55]

Table 2. Hantaviruses, their primary rodent reservoirs and, if applicable, the associated human syndrome. HFRS = Hemorraghic Fever w. renal Syndrome; HPS = Hantavirus Pulmonary Syndrome. * distinct hantavirus species recognized by International Committee on Taxonomy on viruses.

2.2.2. Spillover hosts.

Infection of non-primary hosts (also called spillover hosts, accidental hosts or dead-end hosts) result in a nonproductive infection. Evidence has been found for interspecific spillover of PUUV from its primary host *Myodes glareolus* to *Microtus arvalis* (common vole, [veldmuis] and *Apodemus sylvaticus* (long tailed field mouse, [bosmuis]) [56 57]. PUUV-like RNA has also been detected in *Ondatra zibethicus* (muskrat, [muskusrat]) but the epidemiological implications of this finding are not clear [58]. Tula virus (TULV) has been isolated from *Microtus subterraneus* (european pine vole, [ondergrondse woelmuis]) and *Microtus agrestis* (field vole, [aardmuis]) in the Balkan [59 60]. The role of *M. subterraneus* and *M. agrestis* in TULV epidemiology is unclear.

Spillover to humans can result in profound morbidity and mortality. Human-to-human transmission has only been described once for Andes virus [61] and humans are considered as dead-end hosts as well. Spillover to other animals than rodents has been reported as well, including *Neomys fodiens* (eurasian water shrew), *Sorex minutus* (pygmy shrew), *Sorex araneus* (common shrew), *Crocuidura russula* (greater white-toothed shrew) and *Talpa europea* (european mole) [62 63 64 65 66].

2.2.3. Bank vole population structure and dynamics.

The majority of reservoir hosts for rodent-borne viruses are generalist, opportunistic mammals. In contrast to so called specialist mammals, these species are relatively common, highly fecund, rapidly maturing, highly mobile and are habitat and dietary generalists. These species often take advantage of disturbed conditions, reproducing to very high densities in a short period of time. The bank vole is considered to be such an opportunistic species [67]. For a good understanding of hantavirus epidemiology, a good understanding of bank vole population structures and dynamics is required. Appendix 1 gives an overview of bank vole characteristics that are relevant for hantavirus epidemiology.

Fluctuations in rodent densities in north-western Europe (temperate deciduous broadleaf forest biome[†]) are almost solely related to seed production by broadleaf forests (beech, oak). Peaks in bank vole densities correlate to preceding peaks in seed production (mast years) [68 69]. An abundance of resources can a) improve winter survival, b) elongate the breeding period, c) result in a higher proportion of breeding females and d) induce winter breeding ([7] and reference therein). As a consequence the bank vole population densities will remain high from autumn until next spring. Factors influencing tree seed production will indirectly influence bank vole densities and therefore ultimately the human PUUV incidence (see 8). Paragraph 7 describes the role of rodent population structure and dynamics in hantavirus epidemiology.

In Nordic countries, like Sweden and Finland, fluctuations in rodent densities are predator-driven (taiga biome = subarctic and humid with boreal forest and hardly any broad leave seed production).

[†] Biomes are climatically and geographically defined areas of ecologically similar climatic conditions, such as distinct communtities of plants, animals and soil organisms. Biomes are defined by factors such as plant structures (such as trees, shrubs, and grasses), leaf types (such as broadleaf and needleleaf), plant spacing (forest, woodland, savanna), and climate.

2.3 Clinical aspects

2.3.1. Human

Hantaviruses are the etiologic agents of two distinct human diseases: hemorrhagic fever with renal syndrome (HFRS) in the Old World and hantavirus pulmonary syndrome (HPS) in the New World. Table 2 gives an overview of the to-date recognized hantaviruses and the associated human syndromes.

In Europe, three serotypes are known to cause HFRS, PUUV, Dobrova virus (DOBV) and Saarema virus (SAAV). The incubation period may vary from a few days to 4 weeks [70]. Recently, an unusual long clinical incubation period of 6 weeks has been reported for PUUV [71]. The course of infection varies from subclinical to fatal. The infection is generalised and can affect several organs [70]. HFRS has a complex clinical manifestation characterized by fever, renal dysfunctioning and occasionally acute myopia and haemorrhages. The clinical course of HFRS involves five overlapping phases, *viz*. febrile (fever), hypotensive (shock), oliguric, diuretic (polyuria) and convalscent. However, it is not uncommon for one or more of these phases to be inapparent or absent [64]. The mortality rate varies from 0.1% to 16% depending on the hantavirus serotype [64].

In northern and western Europe, PUUV is the predominant serotype, which causes nephropathia epidemica (NE), the mildest form of HFRS with a mortality rate ranging from 0.1% (Finland)-0.4%(Bashkiria) [70]. An estimated 30% of the PUUV infections lead to disease with serological confirmation [72]. NE usually starts suddenly with fever, intense headache, abdominal and back pains, nausea and vomiting [64]. Renal failure, accompanied by back pain and tenderness, starts around 3-4 days upon the onset of illness and manifests itself in about 50% of the patients by oliguria or even anuria. Hypotension (shock) may develop rapidly at the same time, but occurs only in very severe cases (< 10%). A minority of cases show acute renal failure accompanied by a severe imbalance of the electrolytes. In less than 7% dialysis is required. Ten percent of the cases show haemorragic manifestations like petechiae, melaena and visible haematuria. Severe haemorrages occur in 2% of the patients. Occasionally acute myophia occurs (figure 7, Table 4) [73] [70]. Very rarely severe neurological manifestations have been described [74] [75] [76] [77].

In children, the clinical picture is essentially similar, but often less severe than in adults [78] [79].

Recovery usually begins during the second week of illness, indicated by polyuria. The final convalescence phase to complete recovery can last weeks to months. Longer-lasting complications are very rare [64] [70].

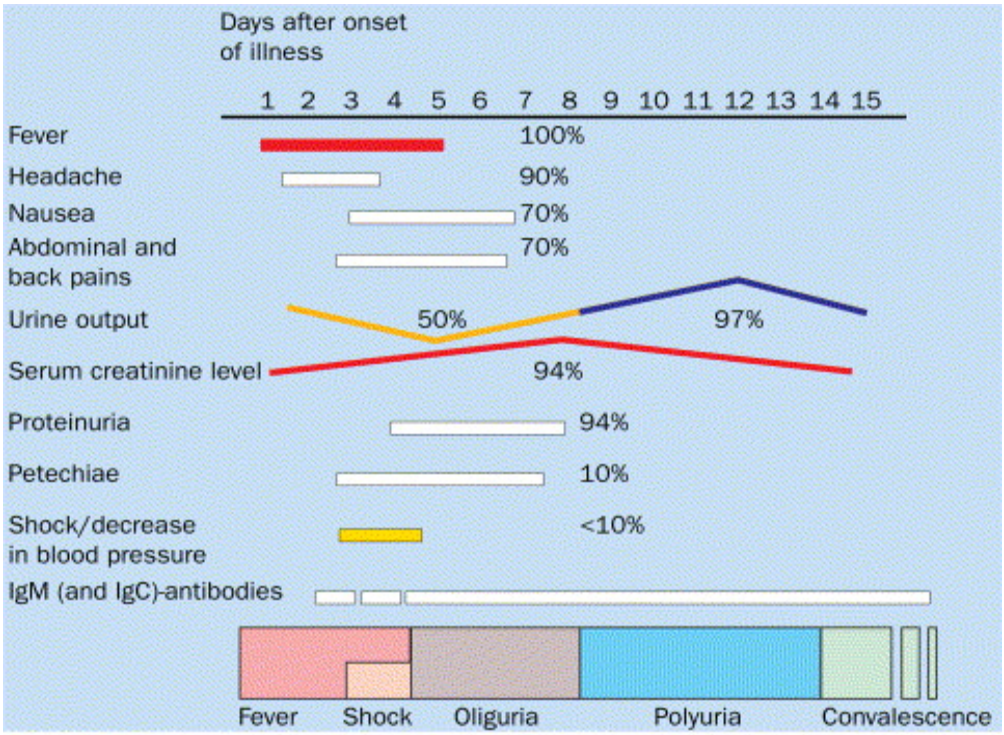

Figure 3. schematic representation of typical clinical course of NE. The five different phases in the clinical course of infection are indicated at the bottom. Percentages (%) are indicative for the incidence of the various symptoms (taken from [70]).

TULV has long been considered as non-pathogenic but recently has been associated with mild HFRS in two cases [80, 81]. TULV and PUUV N-antigens are highly cross-reactive in serological tests [21,70]. If the pathogenesis of TULV in humans is similar to that of PUUV, it cannot be ruled out that of some human infections the hantavirus type might be misdiagnosed.
Immunity against hantaviruses is considered limited to the serotype that has triggered the immunerespons [82,83]. The immunity is considered as life-long as neutralizing IgG antibodies have been detected in covalescent sera several decades upon infection [84]. In Belgium there are patients who are still IgG positive more than 20 years post infection including a veteran who was infected with Hantaanvirus during the Korean war (1951) (P. Heyman, *personal comm*.). Currently no safe and effective vaccine or antiviral treatment against hantaviruses exists [85].

2.3.2. Rodent.

Hantavirus infections in rodent hosts are generally considered asymptomatic due to the million years of co-evolution [86]. In the majority of studies no evidence for clinical illness or effects on rodent reproductivity as a consequence of hantavirus infection is found. Hantavirus infections in their natural, primary host are chronic: the host immune response does not clear the infection and virus replication is persistent. Most interestingly, Kallio *et al*., showed that PUUV infected bank voles had a significant lower overwinter survival probability than seronegative animals [87].
A few papers decribe tissue pathological alterations in the rodent hosts due to hantavirus infection; septal edema within lung tissue and mononuclear cell

infiltrates in portal areas of the liver in *Peromyscus maniculatus* due to Sin Nombre virus infection [88]; lymphohistocytic infiltrates in hepatic portal zones and slightly increased numbers of immunoblasts in splenic red pulp of *Peromyscus leucopus* infected with New York virus [89] insulitis with associated hyperglycemia in pancreas of infected Lewis rats (*Rattus norvegicus*) with SEOV [90].

3. Human diagnostics.

3.1. In general.

The diagnostics of human cases of hantavirus infections are in general based on serology; the detection of IgM and IgG antibodies directed against hantavirus. Viral RNA is detectable in blood or serum from acute PUUV cases in less than two-thirds of the patients [91] and only at a very early stage of infection, making diagnostics based on antigen detection unreliable. Both IgM and IgG antibodies are usually present within 2-8 days of disease onset [92]. In rare cases (<2%) of PUUV infection a delay (up to 5 days after onset of illness) in IgM antibody response has been observed. Therefore a negative IgM response after 6 days upon the onset of illness is considered to rule out a PUUV infection [93]. Most PUUV patients are negative for PUUV IgM 2-5 months after disease onset. IgG antibodies usually remain high for 2-5 months and than gradually decline over 2-3 years. Most patients are still seropositive for PUUV IgG after 2-3 years [92]. The early antibody response is predominantly directed towards the nucleocapsid protein but also against the glycoproteins G1 and G2 [94,95].
The serological cross-reactivity that is observed between different types of hantaviruses reflect their genetic (evolutionary) distances and follow the genetic distances between their primary reservoir hosts ([70] see 2.1). The hantavirus IgM and IgG antibody response can be divided into two groups based on the amount of cross reactivity: 1) DOBV, SAAV, SEOV and HTNV; 2) PUUV, TULV, TOPV and SNV-like viruses [96,97]. Antibodies cross react strongly between DOBV, SAAV, SEOV and HTNV and between PUUV, TOPV and TULV. The cross reactivity of the serological response is very weak or completely absent between PUUV and DOBV/SAAV, especially during the acute phase of infection. Therefore, both antigens PUUV and DOBV/SAAV (or at least antigens representing both serogroups), are to be included in human diagnostics to cover the different types of hantaviruses causing HFRS in Europe [70]. An important consequence of the observed cross reactivities is that the infecting hantavirus type can only be established definitely by neuralisation tests comparing antibody titers with all relevant hantavirus types or by RT-PCR followed by amplicon sequencing. This holds in particular true for the genetically and antigenetically closely related DOBV and SAAV ánd the antigenetically closely related PUUV and TULV. As mentioned above, molecular typing of the infecting hantavirus type is seriously hampered due to the very short viremic stage directly after the onset of disease.

3.2 In the Netherlands.

In the Netherlands human hantavirus diagnostics is offered at three laboratories: Erasmus Medical Centre in Rotterdam; University Medical Centre St. Radboud in Nijmegen; Centre for Infectious Disease control in Bilthoven (RIVM rapport 230071001/2008) an indirect immunofluorescence assay (IFA) or an IgG enzyme-linked immunosorbent assay (ELISA). The IFA (Progen Biotechnik, Heidelberg, Germany) is based on native viral antigen grown in cultured cells infected which Hantavirus (PUUV, HTN or SEOV). Infected cells are fixated on glass slides with are incubated with human sera. To detect the presence of antibodies from both serogroups, both cells infected with PUUV or HTNV are fixed on a multiwell slide and tested. With this technique it is possible to distinguish between antibodies directed against hantaviruses from either serogroup 1 or 2. The ELISA (Focus Diagnostics, Cypress, CA, USA) uses a cocktail of baculovirus expressed truncated nucleocapsid protein of a variety of hantavirus types, f.e. SEOV, HTNV, PUUV, DOBV and SNV. With this technique only the presence of antibodies against hantaviruses can be established. Seropositivity cannot be linked to one of the two serogroups.

In general the discriminatory ability of the diagnostic tests used in the Netherlands is considered to be sufficient under the assumption that only PUUV circulates. A definite typing of the actual hantavirus causing disease is not considered to be necessary. In regions were different serotypes might be sympatric due to overlapping biotopes of the corresponding reservoirs, f.e. Central Europe, determination of the infecting serotype will be more complex. This risk profile shows that human infections with TULV cannot be ruled out and infection with SEOV should also be seriously considered. A more discriminatory diagnostic setup should be implemented both from a medical and epidemiological point of view as these viruses differ significantly in their pathogenicity. It would improve our knowledge about the infecting hantavirus types and their epidemiology in the Netherlands. Reports on the circulation of DOBV in *Apodemus flavicollis* in surrounding countries should be monitored closely to assess the possibililty of DOBV circulation in the Netherlands. In case DOBV infections are considered a serious possibility for the Netherlands, a more discriminating diagnostics is required as DOBV and SEOV belong to the same serogroup with known highy cross reactivity but show differences in severity of illness.

Recommendation.
To improve the human hantavirus diagnostics in the Netherlands. The establishment of more discriminating human hantavirus diagnostics in the Netherlands will enable the exact typing of infecting hantavirus types and related pathogenicity, and improve our knowledge on hantavirus epidemiology in the Netherlands.

Recommendation.
To closely monitore the international developments in hantavirus circulation in rodent reservoirs and human outbreaks, with a focus on the advancement of new types of hantaviruses from adjacent countries, in particular DOBV and SEOV.

4. Human epidemiology.

4.1 In Europe.

In several European Union countries human hantavirus infections have been notifiable for a number of years (e.g. Belgium, Germany, France, Slovenia and Scandinavia). As a consequence the epidemiology of hantavirus infections in these countries has been studied relatively well (Table 3, [4]). In most European countries the incidence of hantavirus infections has increased over the last couple of years [1 2 3 4 5] with severe outbreaks reported for Sweden and Germany in 2007 [98 99]. For the Czech republic and Belgium an unusual number of cases has been reported early 2008 [5 7]. In Belgium a 3-year epidemic cycle abruptly changed into a 2-year cycle in the year 2000 [7]. An extension of the hantavirus endemic areas has been observed for Belgium, France and Germany, including an invasion of urban regions in Germany [100 6].

4.2 In the Netherlands.

In the Netherlands human hantavirus infections became notifiable in december 2008 and until now there are only incomplete datasets on the incidence of human hantavirus infections. In the period 1974-1983 a seropositivity in healthy blood donors in the Netherlands of 0.7% and an overall seropositivity when including populations at risk of 0.9% was found [66]. The Dutch "Virologische weekstaten" suggest that the hantavirus incidence has increased significantly in 2007 in the Netherlands as well (Table 4). Figure 4 shows the geographic spread of the human hantavirus cases in the Netherlands in 2007 and 2008 (till april) as registered by the three diagnostic laboratories in the Netherlands.
An unusual number of cases has been reported early 2008 and a third endemic region besides the known regions Twente and Limburg has surfaced, *viz*. North-Brabant. [101]
For a proper assessment of the risks for human hantavirus infections in the Netherlands, it is necessary to determine the current hantavirus seroprevalence in the Dutch population and the temporal-spatial variations therein. Considering the expected patchy/focal presence of PUUV in areas with bank voles in the Netherlands an option for a cost-effective and "quick-and-dirty" route to map the *current* human risk areas for PUUV infections in the Netherlands would be the assessment of the hantavirus seroprevalence in the Dutch population and the temporal-spatial variations therein. However this will not provide information on the risks for emergence of known and unknown hantaviruses in areas considered free of hantaviruses based on human cases (see 5) nor will it, with the current human diagnostics, provide the necessary insight in circulating hantavirus types and rodent species involved.

Recommendation.
To determine the current hantavirus seroprevalence in the dutch population and the temporal-spatial variations therein. This could f.e. be determined using the PIENTER sera present at the Cib/RIVM[‡].

[‡] For 2009 a screening of a cohort of the PIENER sera is planned. The criteria for the cohort have not been defined yet.

Table 3. Hantavirus cases by the EU, Bosnia-Herzegovina, Norway, Russia and Switzerland as of December 2006 (ENIVD study 2007, taken and corrected from [4]).

Country*	Year when diagnostic was started	Number of cases reported in total by the reference laboratory	Percentage of total cases reported in the European Union	Notifiable disease**	Hantavirus Serotype
Austria	Not available	198	0.60	No	PUUV
Belgium	1981	1856	5.66	Yes	PUUV
Cyprus	2005	0	0.00	No	
Czech Republic	1998	23	0.07	Yes	PUUV
Denmark	1999	0	0.00	Yes	PUUV
Finland	1979	24,672	72.22	Yes	PUUV
France	1987	1,536	4.68	No	PUUV
Germany	2001	1,320	4.03	Yes	PUUV/DOBV/SAAV
Greece	1997	37	0.11	Yes	DOBV
Hungary	1992	302	0.92	Yes	PUUV/DOBV/SAAV
Italy	1991	0	0.00	Yes	None
Lithuania	2000	9	0.03	Yes	PUUV/SAAV
Luxembourg	2000	16	0.05	Yes	PUUV
Netherlands	1994	43	0.13	No	PUUV
Portugal	1990	31	0.09	No	?
Romania	2005	2	0.01	No	PUUV/DOBV
Slovenia	1985	221	0.67	Yes	PUUV/DOBV
Spain	2001	0	0.00	No	None
Sweden	1994	3,516	10.73	Yes	PUUV
Bosnia-Herzegovina	1990	555	***	Yes	PUUV/DOBV
Norway	1990	1,084	***	Yes	PUUV
Russia	1980	89,162 (1996-2006)	***	Yes	PUUV/DOBV/TULV/ HTNV/AMRV/SAAV
Switzerland	2000	1	***	Yes	TULV

* no information obtained for Bulgaria, Estonia, Ireland, Latvia, Malta, Poland, Slovakia, United Kingdom
** hantavirus infection is a -by law- notifiable disease, within 48 hrs after confirmation in the laboratory
*** non-EU Member State.

Table 4. Hantavirus notifications in "Virologische weekstaten" (anonymous).

2004	2005	2006	2007	2008
-	7	8	22	17

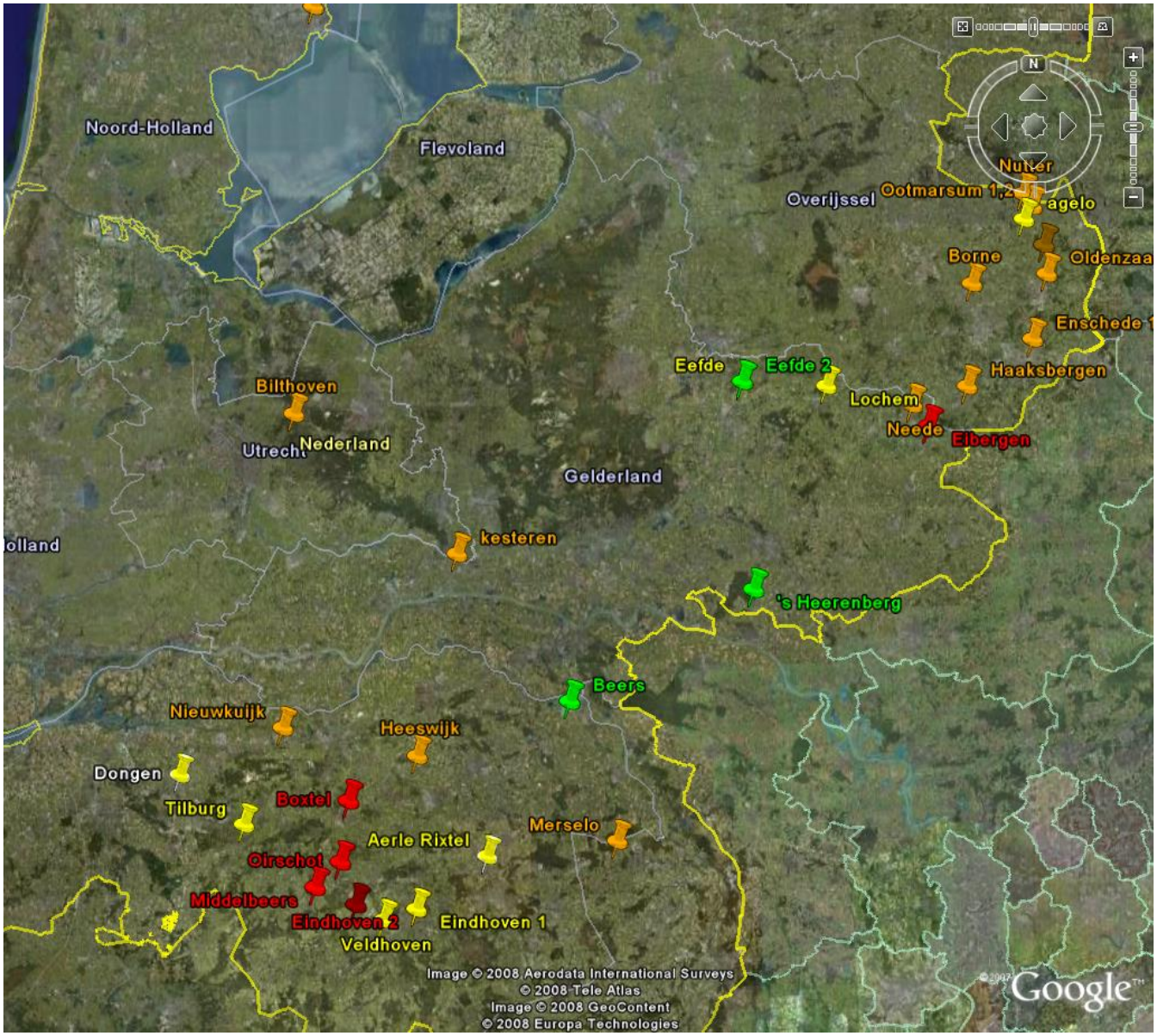

Figure 4. Spatial distribution human PUUV cases in the Netherlands as registered by the 3 diagnostic labs for 2007 and 2008 (till April 2008). Yellow indicates cases identified by the RIVM in 2007; red indicates cases identified by the RIVM in 2008; orange indicates cases identified by Erasmus MC in 2007; green indicates cases identified by Nijmegen MC in 2007. Picture provided by J. Reimerink, LIS/Cib.

5. Geographic distribution.

5.1 Rodent host.

Hantavirus-rodent host relationships are very specific (see 2.2). The geographic distribution of the specific rodent host limits the geographic distribution of a particular hantavirus type. Figure 5 illustrates this correlation between rodent distribution and hantavirus circulation for Puumala virus and its host *Myodes glareolus* in Europe.

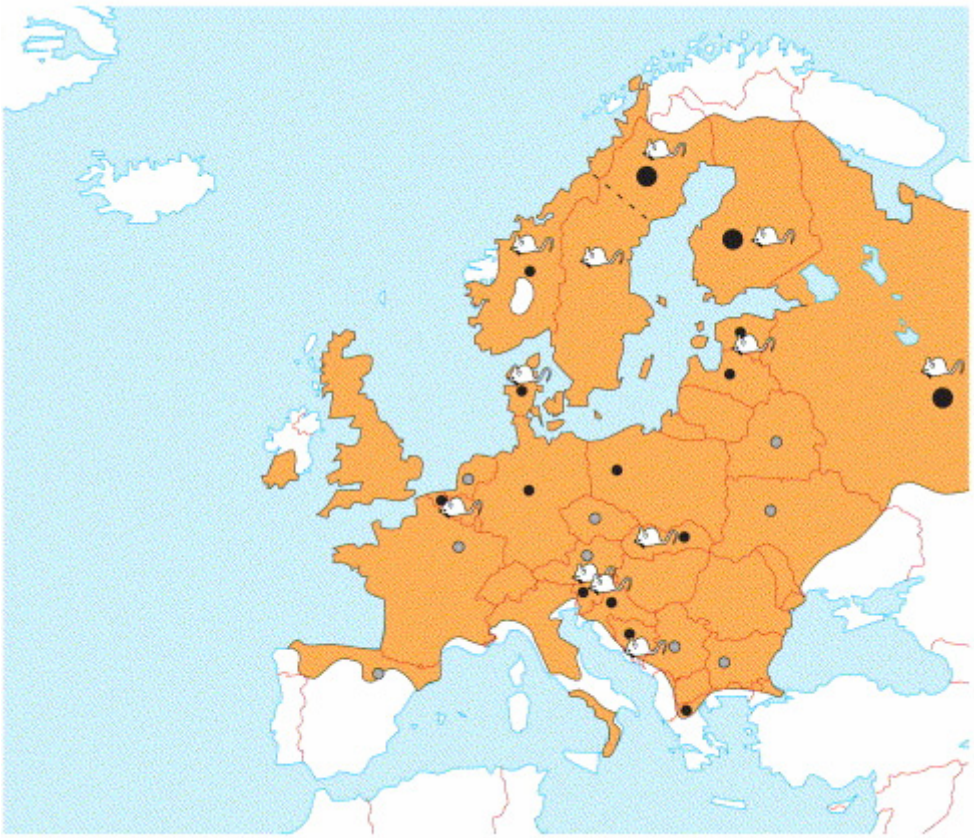

Figure 5. Map of distribution of Puumala hantavirus and its carrier rodent *Myodes glareolus* in Europe. The distribution of *M. glareolus* in Europe is indicated in orange. Rodent figures indicate countries where Puumala virus sequences are available from *M. glareolus*. Grey dots indicate human hantavirus infections caused by Puumala virus as confirmed by serology. Black dots indicate human hantavirus infections caused by Puumala virus as confirmed by cross-neutralisation tests or sequencing of RT-PCR products. Large dots in countries with >200 cases annually (taken from [70]).

As a consequence of the specific hantavirus-rodent host relationship, the geographic distribution of the rodent host can be used to identify locations were specific types of hantaviruses might circulate or emerge [102 103].

In Europe currently six rodent species are recognized as primary rodent carrier for five different hantaviruses (Table 5) [70].

Table 5. Primary carrier hosts and hantaviruses circulating in Europe.

Rodent host	**Virus**
Myodes glareolus (bank vole, [rosse woelmuis])	Puumala
Microtus arvalis (common vole, [veldmuis])	Tula
Rattus norvegicus (Norwayrat, [bruine rat]),	Seoul
Rattus rattus (black rat, [zwarte rat])	Seoul
Apodemus flavicollis (yellow-necked mouse, [grote bosmuis])	Dobrava (DOBV-Af)
Apodemus agrarius (striped field mouse, [brandmuis])	Saarema (DOBV-Aa)

PUUV is the most common hantavirus and is found in most countries of northern-western Europe, including the Netherlands (Reusken *et al*., unpublished results), Belgium, Germany, Austria and France. DOBV has been found in the Czech Republique, the Slovak Republique, Hungary, Albania, Greece, Croatia, Bosnia, Serbia and Russia. SAAV has been found in Finland, Denmark, Estonia, Russia, Germany, the Slovak republic and Slovenia [104]. TULV has been found in the western European countries Belgium, Germany, France, Austria and the Netherlands [81 105,106 107] (Reusken *et al., in press*). SEOV has been found in wild *Rattus norvegicus* in France and in Flanders, Belgium [108 109].

5.2. Rodent hosts in the Netherlands.

In the Netherlands, four types of hantaviruses putatively circulate based on the presence of the associated rodent hosts. Five rodent species known as primary reservoir hosts for hantaviruses can be found in the Netherlands: (a) *Myodes glareolus* (bank vole), host of PUUV, (b) *Rattus norvegicus* (Norway rat) and *Rattus rattus* (black rat), both hosts of SEOV, (c) *Apodemus flavicollis* (yellow-necked mouse), host of DOBV and (d) *Microtus arvalis* (common vole), host of TULV.

Myodes glareolus (bank vole) is commonly present in the Netherlands with exception of the islands of Goerre-Overflakkee, Vlieland and Ameland (Figure 6).

Myodes glareolus

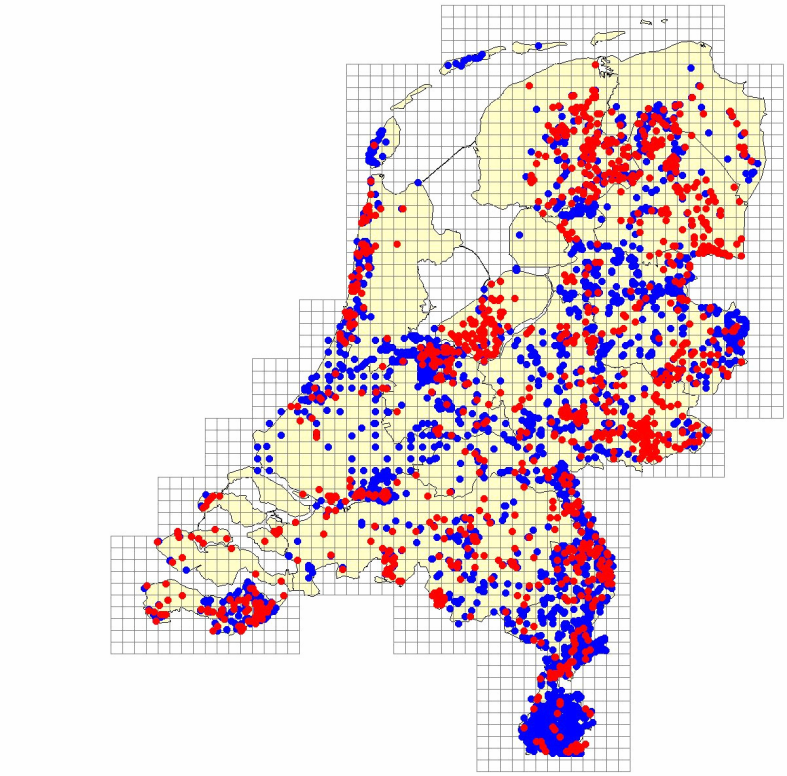

Figure 6. Distribution of *Myodes glareolus* in the Netherlands. Blue dots represent observational data, red dots represent *M. glareolus* remains found in owl pellets (D. Bekker, VZZ *pers. comm.*).

Microtus arvalis (common vole*)* is commonly present in the Netherlands with exception of the islands of Texel, Vlieland and Terschelling (figure 7).

Microtus arvalis.

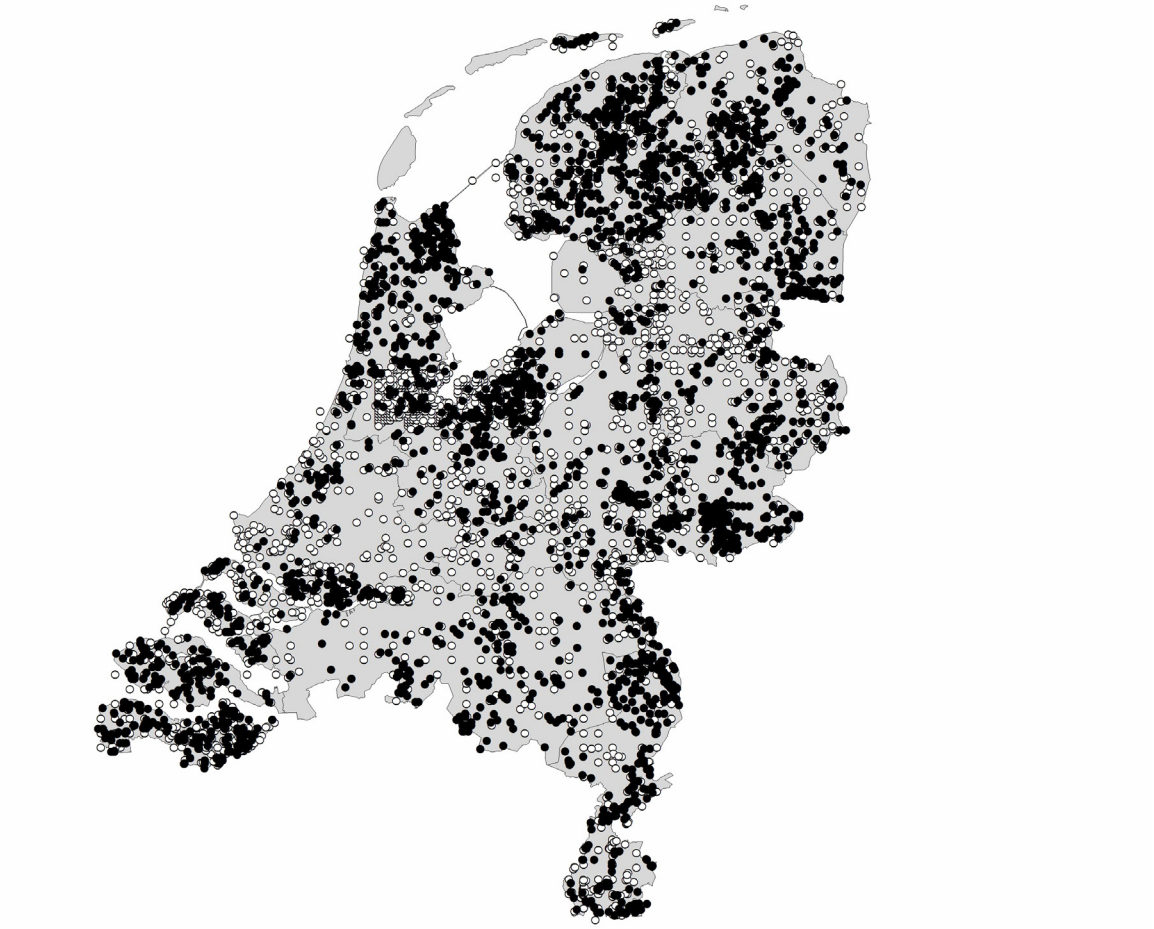

Figure 7. Distribution of *Microtus arvalis* in the Netherlands. White dots represent observational data, black dots represent *M. arvalis* remains found in owl pellets (D.Bekker, VZZ *pers. comm*.).

Rattus norvegicus is commonly observed in the Netherlands (figure 8). However its presence at the various locations is subjected to change as the Norway rat is controlled by professional rat controllers.

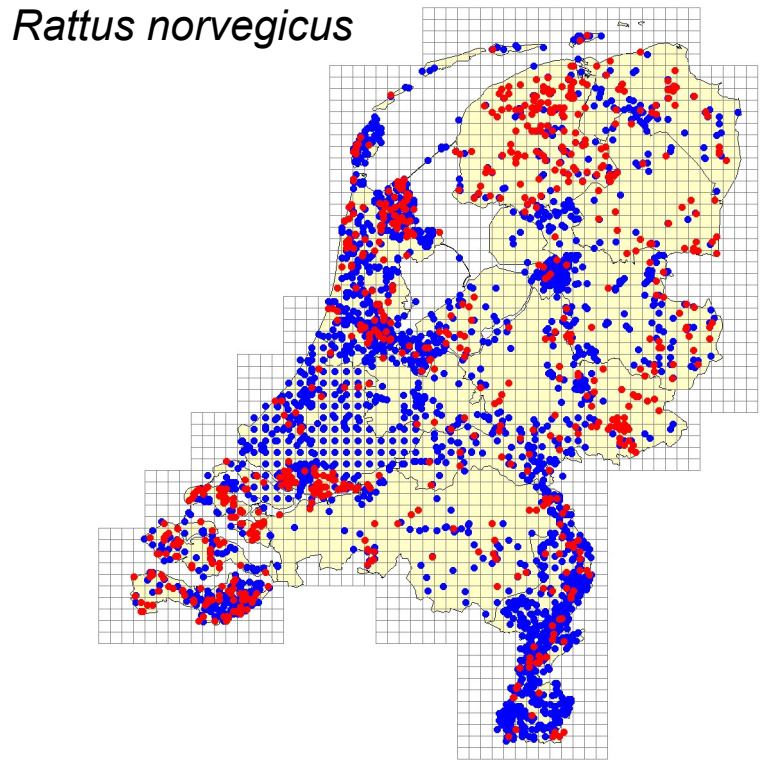

Figure 8. Distribution of *Rattus norvegicus* in the Netherlands. Blue dots represent observational data, red dots represent *Rattus norvegicus* remains found in owl pellets (D.Bekker, VZZ *pers. comm*.).

Rattus rattus is most commonly present in the provinces of North-Brabant and Limburg but rarely observed in the rest of the Netherlands (figure 9). In 2008 an increase in *Rattus rattus* populations has been reported for the Southern provinces Limburg and North-Brabant. *Rattus rattus* populations are subjected to immediate extermination by professional rat controllers.

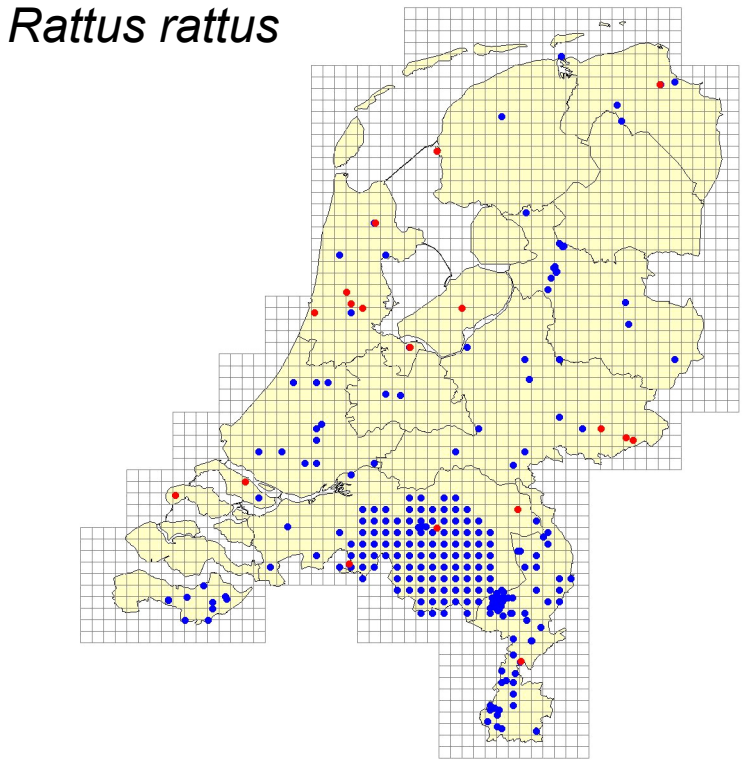

Figure 9. Distribution of *Rattus rattus* in the Netherlands. Blue dots represent observational data, red dots represent *Rattus rattus* remains found in owl pellets (D.Bekker, VZZ *pers. comm*.).

Apodemus flavicollis is only observed at three locations in the Netherlands, in the woods aroud Winterswijk in the province of Gelderland and at two locations in the most southern part of the province of Limburg (D. Bekker *pers. comm*.). However, very recent data collected in August/ September/ October 2008 indicate that the *Apodemus flavicollis* populations in the Netherlands seem to increase at the moment in the border regions with Germany, Luxemburg and Belgium and more locations are expected to emerge (D. Bekker, VZZ, *pers. comm*.).
Other rodent species of interest but with an unknown contribution to hantavirus epidemiology are *Microtus agrestis, Microtus subterraneus* (both TULV) and *Apodemus sylvaticus* (PUUV). *Apodemus agrarius* is not present in the Netherlands.

Based on the geographic distribution of these rodent hosts in the Netherlands, the following can be considered:

a) a nationwide circulation of PUUV, at this point with exception of the West Frisian islands Vlieland and Ameland and the island Goeree-Overflakkee in the province South-Holland.

b) a nationwide circulation of TULV, at this point with exception of the West Frisian islands Texel, Vlieland and Terschelling.
c) circulation of SEOV at locations were *Rattus norvegicus* or *Rattus rattus* are present.
d) circulation of DOBV at locations were *Apodemus flavicollis* is present.

5.3 Hantavirus within geographic range host.

The geographic range of the hantavirus infected rodent hosts determines the area in which transmission to humans can occur. It is important to note that human hantavirus cases are often more spatially restricted than the geographic distribution of the reservoir host, e.g. NE cases caused by PUUV in Western Europe. However, it is not justified to assume that hantaviruses only circulate in areas of human disease as in general only these areas are subjected to research, like in the Netherlands. In the USA, Sin Nombre hantavirus was found regularly in its reservoir host in a wide region, including cities, while the 1993 outbreak was very local [110 64]. PUUV circulates in bank voles in some areas in Belgium where most human cases were believed to be imported from elsewhere [7].

A recent model study showed that the potential risk for public health of hantavirus emergence may be greater than believed based on the distribution of human cases. Disturbances in stable demographic patterns in rodent populations could lead to the emergence of known and unknown hantavirus infections in the human population in areas considered free of hantaviruses (see 7, [110]). As a measure for the human transmission risk that could incur following sudden changes in bank vole demography, it is important to assess the general distribution of PUUV circulation in bank voles in the Netherlands. In some areas PUUV circulation might indeed be more restricted than the geographic distribution of the bank vole. The lower PUUV incidence in northern Belgium in comparison to southern Belgium was related to the more pronounced forest fragmentation in northern Belgium [7]. Bank voles are very sensitive to deciduous forest patch sizes due to the low dispersion possibilities. PUUV simply might not have had the chance to reach certain patches regardless of whether the local environmental and bank vole conditions would allow it. Based on the high rate of forest fragmentation in the Netherlands, a very patchy/focal presence of PUUV in areas with bank voles can be expected. The further development of the “ecologische hoofdstructuur” (EHS) could increase the spatial distribution of rodent hosts and hantavirus circulation therein.

Another complicating feature is the observed spatial and temporal variations in infection rates in any given population which can vary between high to low or even absent [111]. Except during years with exceptionally high rodent densities, infected animals occur in foci [111]. High density of plant coverage was shown to give a higher probability for seropositivity in bank voles [112] as was an increased deciduous forest patch size [113] This implies that endemic areas should be monitored to follow trends in time to get more insight in the dynamics of rodent-borne hantavirus infections in the Netherlands.

5.4. Hantavirus in rodent hosts in the Netherlands.
The currently known PUUV endemic regions in the Netherlands, identified based on PUUV-seropositive patient numbers, are Twente (province Overijssel), South-Limburg and North-Brabant [66 114 115 101]. See figure 4 for the spatial distribution of human PUUV cases in the Netherlands in 2007 and the first 4 months of 2008. In the past a few bank voles positive for "PUUV-like" antibodies were found in Arnhem (1984, Gelderland), Velsen (1989, North-Holland), Volthe (1989, Twente), De Lutte (1989, Twente), Rossum (1993, Twente) and Boekelo (1993, Twente) [66]. Current research in 2007 and 2008 provided the first genetic evidence for the circulation of PUUV in bank voles in the Netherlands. PUUV antibodies and RNA were detected in bank voles in Nutter (2007/8, Twente), Middelbeers (2008, Brabant), Wintelré (2008, Brabant), and Oirschot (2008) (Reusken *et al*., unpublished results). The current data on the spatial distribution of hantavirus circulation in the Netherlands is too limited for risk assessment purposes. It is essential to assess the general distribution of PUUV in bank voles in the Netherlands. Additional field inventories are necessary.

In 1989 a common vole was found seropositive for "PUUV-like" antibodies [66]. Because common voles are both primary host for TULV and spillover host for PUUV, and TULV cross reacts in PUUV serological tests, it is unclear whether this common vole was positive for TULV or PUUV. Research in 2007 gave the first genetic evidence for the circulation of TULV in common voles in the Netherlands. TULV antibodies and RNA were detected in common voles in Nutter (2007, Twente), Hamster reservaat Stibbe (2007, Limburg) and Zaandam (2008) [116] . For risk assessment purposes, an inventory of the circulation of TULV in common voles is necessary. In addition TULV has been isolated from *Microtus subterraneus* and *Microtus agrestis* in southern Europe [60 59]. *M. subterraneus* and *M. agrestis* are present at several locations in the Netherlands. Its role in hantavirus circulation should be investigated as well. Currently, it is not known whether there are human cases of TULV infections in the Netherlands. To determine the true prevalence of TULV antibodies in humans, more differentiating studies/diagnostics in humans are necessary (see 3).

SEOV has been found in wild *Rattus norvegicus* in Belgium (Flanders) and France [108,109]. Sofar no evidence for wild rat-related human SEOV infections in these countries have been reported although this has not been investigated thoroughly. Laboratory related infections are reported from the Netherlands, Belgium, France and England. No information on the circulation of SEOV in *R.rattus* and *R.norvegicus* in the Netherlands exists. Regarding the presence of SEOV in wild rats in France and Flanders, circulation of SEOV in *Rattus sp*. in the Netherlands cannot be ruled out. For risk-assessment purposes it is necessary to monitor the putative circulation of SEOV in *R.norvegicus* and *R.rattus* in the Netherlands, especially with the observed increase in local *Rattus sp*. populations.
SEOV causes mild HRFS and the severeness of its symptoms might resemble a PUUV infection. To actually identify a human SEOV infection in the Netherlands differentiating diagnostics are required (see 3).

DOBV circulates in *Apodemus flavicollis* in the eastern regions of its geographic range, including east Germany. Circulation of DOBV in *A. flavicollis* in the

Netherlands has not been investigated: *A.flavicollis* is an endangered (red list species) in the Netherlands; currently only three locations with *A. flavicollis* populations are known. There is no evidence for patients with severe HFRS in the Netherlands which would support the possibility of DOBV circulation and justify analysis in *A.flavicollis*. Reports on the circulation of DOBV in *A. flavicollis* in surrounding countries should be monitored closely to assess the possibililty of DOBV circulation in the Netherlands. In case DOBV infections are considered as a serious possibility for the Netherlands, discriminating diagnostics in human cases are definitely required as DOBV and SEOV belong to the same serogroup with known high cross reactivity.

Several studies report *Apodemus sylvaticus* with positive PUUV serology [111] [117]. This has also been observed in the Netherlands (Reusken et al., unpublished results). No hantavirus sequences have been isolated from this species using current knowledge on hantavirus genetic structure. This, together with the report of borderline ELISA results for HTNV and DOBV in *Apodemus* sp. might suggest the presence of a still unknown strain in this species [111]. Further research is necessary.

Recommendation.
To expand our knowledgde on hantavirus circulation in putative reservoir hosts in the Netherlands for risk assessment and disease prevention purposes.
As a measure for the human transmission risk that could incur following sudden changes in rodent population structures and densities, it is important to assess the general distribution of hantavirus circulation in rodents in the Netherlands. Endemic areas should be monitored to follow trends in time to get more insight in the dynamics of rodent-borne hantavirus infections in the Netherlands. Field studies should not be limited to *M. glareolus* and *M. arvalis* , but should include *Rattus* sp., *Apodemus* sp. and other *Microtus sp*.

6. Transmission routes.

6.1 Rodent-to-rodent.

Hantaviruses are shed in saliva, urine and feces of their primary reservoir hosts. Intranasal inoculations of colonized bank voles with saliva, urine or feces of experimentally infected bank voles, were all infectious [118]. Transmission occurs horizontally either *directly* through aggressive behaviour/ sexual contacts , or *indirectly* through inhalation of aerosolized virus particles from a contaminated environment, e.g. through communal nesting [119] [120] [121] [122] [123]. The positive correlation between seropositivity and age of rodent hosts, as observed for wild-trapped bank voles in several studies, is indicative for the importance of horizontal transmission of the virus among rodent populations [124].
Frequent contacts between sexually mature bank voles during the breeding season are generally thought to be critical to hantavirus transmission. Studies on the dynamics of PUUV infection in wild bank vole populations in the Ural mountains showed an increased rate of virus transmission during the period of high reproductive activity [123]. The role for biting incidents (aggressive or grooming behaviour) in virus maintenance in rodent populations is illustrated by the observation that with experimentally infected, colonized bank voles the

highest transmission rates occur when virus shedding in saliva peaks. For an efficient transmission via saliva indirect contacts are not likely as the amount of saliva shedded into the environment is very low [125]. A field study in Belgium showed that aggressive behaviour plays an important role in PUUV transmission during the breeding season. The proportion of injured animals and seropositivity were higher in breeding males and females than in other adults, especially at the end of the breeding season [112].
In addition, model studies indicate an important role for indirect transmission through the environment on virus persistence in fluctuating bank vole populations [126]. Communal nesting is a known overwintering strategy for bank voles and might facilitate the persistence of PUUV during the non-breeding season [127]. The efficiency of this indirect transmission route strongly depends on microclimatic (e.g. temperature, humidity) and chemical parameters (e.g. pH) in the soil, so called virus ecology. Hantaviruses are susceptible to UV-light, high temperatures, acidic and dry conditions. PUUV shows a prolonged survival outside the rodent host. PUUV remained infectious in bank vole cage beddings for 12-15 days at room temperature. In cell culture supernatants, both PUUV and TULV remained infectious for 5-11 days at room temperature and up to 18 days at 4 ˚C. The viruses were inactivated after 24 hours at 37 ˚C [128]. Under sufficiently humid conditions, infected urine mixes with soil water and the virus spreads over a small volume of forest litter. These conditions favour virus conservation as the virus remains in hydrosolution and is protected from direct UV-light and heat [126]. Verhagen and coworkers described that the presence of infected specimens in low-density bank-vole populations is related to humid environments [129]. It has also been reported that the seroprevalence for PUUV in rodents trapped on north-facing slopes is higher than that in rodents trapped on south-facing slopes. It was suggested to be due to the higher humidity on north-facing slopes[57]. Tersago and coworkers observed that areas with low temperatures in winter and summer and high precipitation in spring generally show a high seroprevalence and absolute number of infected specimens [7].

Both direct and indirect transmission routes are involved in hantavirus dynamics in rodent populations. The relative importance of these two modes of transmission will vary intra-and interannually, depending on hantavirus species, host-population structure/dynamics and the environmental conditions [128] [118]. The presence of other rodents has also been suggested to influence the transmission dynamics, e.g. by influencing the reservoir host densities and their spatial distribution and social behaviour. Tersago et al. (2008), found indications for a dilution of the prevalence of PUUV in a bank vole population, dependent on the relative proportion of spill over host *Apodemus sylvaticus* present [7].

6.2 Rodent-to-human.
In addition to the role of the indirect transmission route in virus transmission among the reservoir host population, it is also considered to be the main route of infection for accidental hosts like humans [70]. A synchrony of infection rates in rodent reservoirs and humans over 3-year epidemiologic cycles is observed. This temporal correlation between infection rates in rodents and humans is indicative for a common mode of infection. As a consequence, factors affecting the efficiency of the indirect transmission path directly and indirectly (through

influencing the prevalence in the reservoir host) influence the transmission risks for humans [57] (see 6.1 and 8).

7. Hantavirus dynamics in rodents.

7.1 Hantavirus dynamics at rodent population level

A direct correlation between bank vole densities, demography, population infection rate and the transmission risk of PUUV to humans exists. The infection rate of bank vole populations is directly correlated to bank vole population structure and densities [130]. Factors influencing these parameters, so called host ecology, will influence the human transmission risk (see 8). To understand the dynamics of PUUV infections in bank voles at the population level, it is necessary to understand bank vole population structures and dynamics (see 2.2.3) and hantavirus dynamics at the individual rodent level (see 7.2).
The chances of infection for bank voles are the highest during the peak of the bank vole population cycle, assumingly because the likelihood of exposure to hantaviruses increases with increasing population densities. [127].

The capacity of hantaviruses to avoid extinction during low-density periods in bank vole populations depends on reservoir host population dynamics and on the specific characteristics of the specific host-virus interaction. The most important feature of the PUUV-bank vole relationship is that the virus does not affect the bank vole population structure and dynamics.
Three hypothesis about hantavirus survival during periods of low-densities in bank vole populations are discussed in literature [126].

1. Persistent infection hypothesis: hantaviruses are shed chronically by infected rodents and do not cause host mortality or affect fecundity. Infection of long-lived specimens provide a continuous virus reservoir which sustains the virus during periods of low host densities. This strategy is supported by studies from Olsson *et al*. showing that the presence of long-lived animals (animals > 11 months of age) is critical for the persistence of hantavirus circulation in a bank vole population. Localized absence of PUUV coincided with the absence of overwintered specimens during low population densities [127]. Capture-mark-recapture studies suggest that a crucial threshold in bank vole density exists for the maintenance of an enzootic cycle [111]. Tersago *et al.* also found indications for a density threshold below which PUUV does not occur; this threshold density for maintenance of an epizootic cycle will vary annually based on local bank vole population and environmental conditions [7].
2. Dual host tropism hypothesis: hantaviruses can survive in asynchronous populations of a reservoir host living in sympatry with the primary host. Primary hosts can become infected from these secondary reservoir hosts when their population density increases again. This strategy is supported by documented host switches in the evolutionary history of rodent-hantavirus combinations [131]. However for PUUV no evidence for the existence of such a dual host tropism is found. The documented presence of PUUV antibodies in *A.sylvaticus* and *M. arvalis* appear to be a consequence of a spillover event rather than a situation of a second reservoir host [56,111].

3. Indirect transmission hypothesis: virus survival outside the host could permit virus transmission without the actual presence of the infectious host or during a temporal loss of the infection in the host population. Epidemiological and model studies show that indirect transmission through the environment significantly increases the probability for PUUV to persist during low-density periods of bank voles. Even a low survival rate of PUUV outside the rodent was already sufficient to decrease the risk of virus extinction. The efficiency of this indirect transmission route depends strongly on microclimatic and chemical parameters in the soil/forst litter (see 6).

7.1.1 Age and sexual maturity of bank voles.

The infection rate of bank voles depends on age and sexual maturity of the animals. Olsson *et al* observed that age is the demographic factor with the highest influence on the probability of PUUV seropositivity [127]. The seroprevalence of PUUV antibodies in wild bank voles increases with an increased age of the voles [124 111 127 7]. In general age is an important epidemiologic feature for horizontally transmitted pathogens because the chance of exposure to these pathogens generally increases with age. The greatest proportion of seropositive animals is observed among overwintered, heavier males. This gender effect is not due to increased aggressive behavior of the males as bank vole males do not defend territories but usually have larger and frequent overlapping home ranges. Territorial behavior is solely attributed to bank vole females[132 133]. Breeding males travel over more than one female territory and will frequently visit nests of other animals and territorial “excretory points”, resulting in an increased risk of virus exposure [123 111,112 127 134]. Other studies observe no gender effect in seropositivity [7]. Besides roaming males, bank vole territories/ populations are connected through juveniles who disperse to find new breeding territories and to reach sexual maturity (see 2.2.3). During dispersal juveniles cross several adult territories and explore a greater area than at any other stage of their life. In addition, dispersing juveniles pay particular attention to urine scents, deposited by other individuals to mark territories. As a consequence juveniles have an increased probability of exposure to the virus [126 135]. A field study in Belgium showed that seroconversions occurred more frequently in animals that had moved longer distances from their capture original point [112]. Dispersal is an essential feature for hantavirus persistence in rodent populations as hantaviruses do not persist in rodent populations when the dispersal rate is set to zero in model studies [126] This modeled role of bank vole dispersal and mobility in hantavirus transmission was supported in a field study in the French Jura region [134] and southern Belgium [112].

7.1.2 Immune status and kinship of bank voles.

Nonimmune voles of any age appear equally susceptible to PUUV infection. Therefore the probability of acquiring an infection increases with age. Seroconversion is more frequent during periods of high reproductive activity among voles. Bank vole populations include young animals with maternal immunity to PUUV. These animals remain resistant to PUUV infection for 2- 3.5 months. Non-immune animals generally become seropositive during the second month of life, maternal immune animals become infected on average 30-45 days

later [123]. Juveniles usually disperse from their natal sites before the disappearance of the maternal antibodies. Therefore, mother-to-progeny transmission is highly unlikely in the pre-dispersal period. PUUV is preferentially transmitted among relatives. Whether this is due to specific bank vole behaviour or to similar genetic backgrounds of relatives is not known [134].

7.2 Hantavirus dynamics in individual rodent host.

Studies on the dynamics of PUUV infection in wild bank vole populations in the Ural mountains showed that there is a peak in virus accumulation and shedding of the virus from the voles during the first month upon infection. The incubation period is short in comparison to the bank vole life expectancy. Although antibodies persist throughout a lifetime, the intensity of virus reproduction, the frequency of horizontal transmission and the accumulation of PUUV antigen considerably decreases in time [123].
PUUV antigens in experimentally infected, colonized vank voles are detected in several organs and tissues, including lungs, kidneys, liver, spleen, salivary glands, brown fat and urine [62,125] . Experimental infection of bank voles by subcutaneous injection with PUUV showed that virus is shedded with peak levels 11-28, 14-21 and 11-28 days post infection in saliva, urine and feces respectively. The latest point of detection was 84, 44 and 44 days post infection respectively. The bank voles were viremic untill 133 days post infection [118].

8 Risk factors for human disease.

The PUUV (and hantaviruses in general) transmission risk for humans depends on a combination of a) host ecology, b) virus ecology and c) human behavior/condition (figure 10).

a) Host ecology involves environmental factors related to bank vole population densities and structure, *viz*. land-surface attributes, landscape configuration and climate. Climatic factors control directly the rodent population dynamics by influencing the winter temperature-dependent survival rate or indirectly by influencing vegetation growth, snow cover and food supply, f.e. mast production.
b) Virus ecology involves environmental factors related to virus survival outside the rodent host, *viz*. level UV-exposure, winter temperature, soil pH and soil moisture. Virus ecology influences the human transmission risk directly and indirectly (through influencing the prevalence in the reservoir host) through the indirect transmission route.
c) Some human activities will be associated with a close contact with the host habitats and thus increase the likelihood of human-host contacts or with a close contact with areas that support virus survival [130,136].

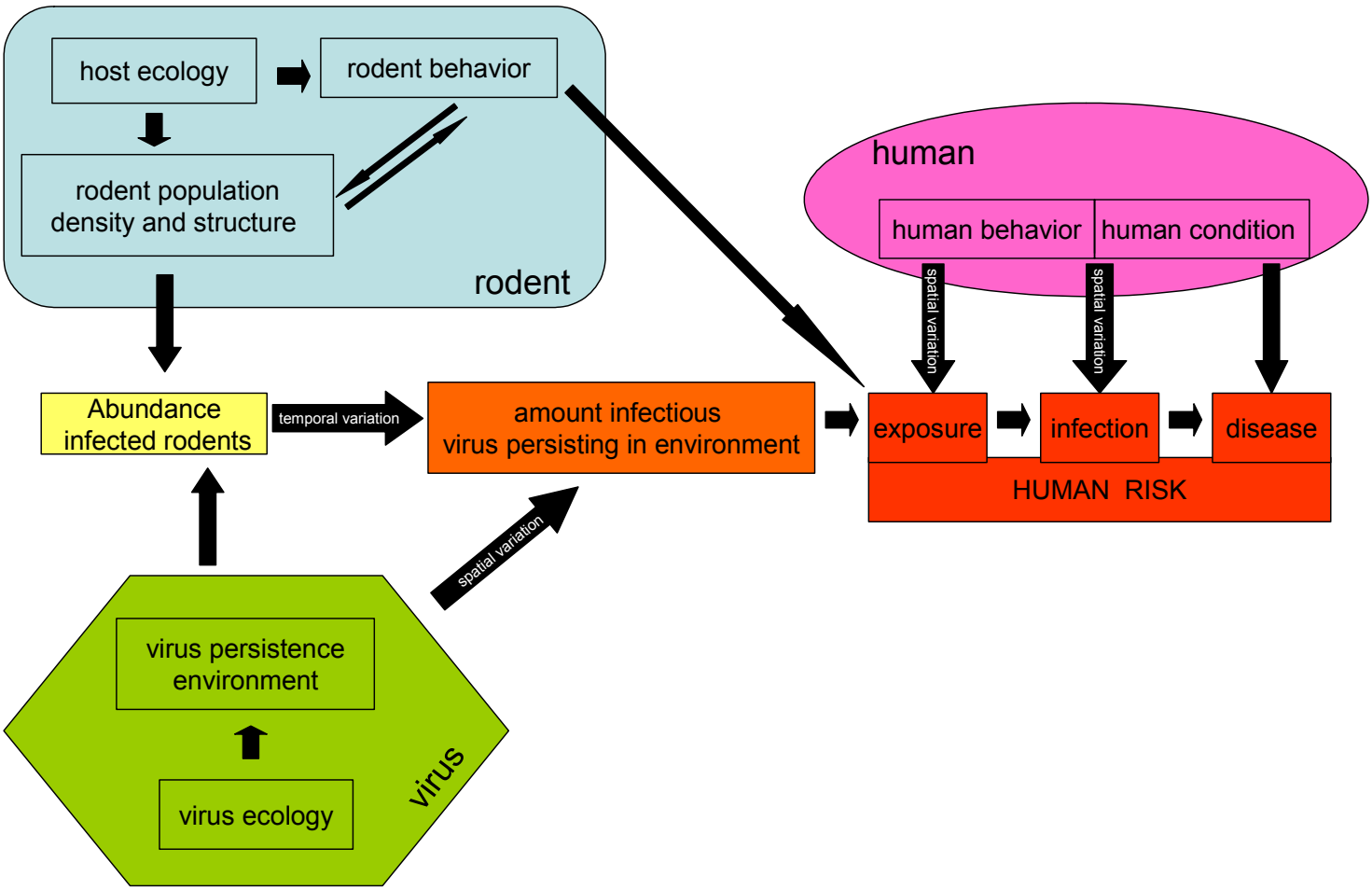

Figure 10. Flow chart factors influencing the human risk for hantavirus infection.

It is generally accepted that the spatial variation in human PUUV infections is predominantly determined by virus ecology in combination with human activities. The temporal variation in human PUUV infection risks is predominantly determined by the abundance of infected bank voles at a given time, which is influenced by both virus and host ecology (figure 10). The latter is supported by the observed correlation between peaks in bank vole population densities and outbreaks of human cases [7,8,20,72,78,111,124,137,138].

Linard *et al*. concluded that above a threshold abundance of bank voles, virus ecology will better predict the NE incidence in humans than host ecology, in particular the degree of soil moisture and the maximal temperatures from the preceding winter. This implies that beyond this threshold an increase in the population size does not necessarily lead to an immediate increase in risk for human transmission [130]. Sauvage *et al*., 2007 also observed that virus ecology greatly influences the transmission risks for humans [110].

Homogeneous high seed production of both beech and oak correlates to an increased incidence of NE in Belgium (year 0). In addition, rather cold and humid summers in year -3, elevated average summer temperatures in year -2 and warm autumn conditions in year -1 consititute a direct link to increased human PUUV infection risk in year 0 [7,8]

Bank voles are highly sensitive to forest fragmentation due to the low dispersion possibilities. This lack of migration might play a significant role in eradication of PUUV from an isolated forest patch [7].

A model study by Sauvage *et al* shows that in order to evaluate the risks for virus transmission to humans, host density and infection rate are not the most important parameters but the speed of the host population increase that allows (or not) for a sufficiently high density of newly infected bank voles for human infection is. This model is based on the observation that infected voles show a peak in virus excretion during the first month upon infection whereas during the chronic state of infection much less virus is excreted into the environment (see 7, [110 62]). Sauvage and co-workers discuss that pathogens may persist in host reservoirs for very long periods below the threshold for cross species transmission. A sudden change in the demographic pattern of the reservoir might lead to a rapid increase in host density which can result in a peak excretion of the pathogen by these newly infected hosts. Viral excretion could reach threshold levels and potentially cause the emergence of human cases. This would explain the observed distribution of NE outbreaks, which coincides with bank vole populations that show multi-annual fluctuations in rodent densities. Therefore, potential areas for hantavirus emergence should be greatly extended (see 5). Disturbances in stable demographic patterns in rodent populations could lead to the emergence of known and unknown hantavirus infections in the human population.

In addition to host ecology, virus ecology and human behavior, rodent behavior was suggested as fourth determinant for human transmission risk in Sweden [98,139]. An increase in NE cases was observed in the year 2007 that was preceded by exceptional mild weather in December 2006. There was no or little snow and hard ice cover in the coastal area of Northern Sweden and higher than average temperatures were observed. Snow cover in winter is essential for bank vole survival (host ecology) as it hides the voles from predators and the cold and offers access to the food below [140]. In the absence of snow bank voles might seek refuge for predators in human dwellings thereby increasing the exposure

risk for humans. Since in western Europe bank vole populations dynamics are more food-driven than predator-driven and snow coverage is less common than in northern Europe it seems unlikely that this correlation between snow cover and increased human exposure is relevant for the Netherlands. However, the existence of factors, that influence rodent behavior (f.e. search for food in human dwelings) leading to increased human exposure, should be kept in consideration.

Risk factors identified in case control studies in literature:
1. land cover. Human PUUV incidence rate is higher when urbanization is low and the proportion of broad-leaved forests is high [130,136].
2. land use. Land use determines the degree of exposure of people to infectious environments. Known risk behaviour are: observing rodents or rodent droppings, farming, harvesting, animal trapping, handling wood (cutting, fetching, carrying), exposure to dust/earth in forest especially off-tracks (work or leisure-related), reopening/entering/cleaning rodent-infested buildings, gardening/digging earth, visiting forest shelters [2,141 142 143 144].
3. settlement characteristics *viz*. the proximity between habitation and forest [2]; For France/Belgium living < 50 m from forest [143]; for Germany living <100 m from a forest [100].
4. Profession/socio-economic status. In Belgium, income was negatively correlated to disease incidence. PUUV infections in particular affected people in certain socio-professional categories associated with low incomes, like forest workers and farmers. Professions at risk: farmers, forestry workers, construction workers (especially when renovating old buildings or working at construction sites near forests), military, animal trappers (both for pest-control and biology studies) [66,100,141,143].
5. being male, smoking cigarettes [145].
For the Netherlands case-control studies have not been performed. Risk factors in the Netherlands are not expected to be different from those identified in other case control studies. However it remains important to assess (specific) local risk behaviour in clusters and isolated cases of NE [8].

9. Hantavirus infection and climate change.

Through aspects of host ecology, virus ecology and human behaviour the incidence of hantavirus infections is partly climate dependent. Clement *et al*., related the increased hantavirus incidence observed in Belgium in recent years to global warming. They observed that both summer and fall temperatures have been rising to significant higher levels in recent years explaining the continiously high incidence rate of human infection since 2005. They predict that due to global warming nephropathia epidemica may become a highly endemic disease in Belgium and countries with comparable disease mechanics (f.e. overall presence of bank voles, temperate deciduous broad-leaf forest biome with more frequent mast years), which includes the Netherlands [8]. However more factors than climate alone will contribute to the overall effect on hantavirus incidence in Europe in the coming decades [145]. See also chapter 10.

10. Early warning.

Homogeneous high seed production of both beach and oak is closely related to an increased incidence of NE in Belgium. Based on this observation and the known correlation between both bank vole abundance/high seed production and bank vole abundance/human PUUV infection risk, it can be concluded that high seed production by beech, oak or both in autumn (year -1), can be regarded as an early warning tool for public health policy makers. Food production in autumn should be monitored and measured to assess human risks in the following year (year 0).
Furthermore, rather cold and humid summers in year -3, elevated average summer temperatures in year -2 and warm autumn conditions in year -1 consititute a direct link to increased human PUUV infection risk in year 0 [7,8]. This in combination with the mast production should be considered as early warning indicators for NE outbreaks in Belgium. This can be extrapolated to neighbouring countries, including the Netherlands as experts believe that the NE mechanics will be fundamentally the same in these countries as they are in the same biome and have a similar documented presence of the bank vole [7,8].
Sauvage *et al*. [110] observed that beyond a threshold abundance of bank voles, virus ecology will better predict the NE incidence in humans than host ecology, in particular the degree of soil moisture and the maximal temperatures from the preceding winter. This implies that beyond this threshold an increase in the population size does not necessarily lead to an immediate increase in risk for human transmission.

Based on these observations and the research on factors influencing virus persistence in the environment, it is feasible to set-up a systematic early-warning system for the timely detection of public health risks due to the abundant presence of hantavirus infected rodents.
Indicators for high virus persistence in the environment and for high rodent densities and virus circulation therein (involving parameters concerning host ecology and virus ecology), should be identified further and continuously monitored. As the bank vole and other rodents/insectivores are known reservoir hosts for other emerging pathogens in the Netherlands and Europe, including *Toxoplasma spp., Babesia spp., Gardia spp. Cryptosporidium spp. Mycobacterium spp. Borrelia spp., Echinococcus multilocularis*, tick-borne encephalitis virus, Ljungan virus, Hepatitis E virus and cowpoxvirus, the predictive factors for hantavirus risks should be integrated preferably in a general rodent monitoring programme.

Recommendation. To set-up a systematic early-warning system for the timely detection of public health risks due to the abundant presence of hantavirus infected rodents. Indicators for high rodent densities and virus circulation therein, involving parameters concerning host ecology and virus ecology, should be identified and continuously monitored.

Recommendation.
To set-up a general systematic early-warning system from a broader perspective including the risks of high rodent densities for public health in general, animal health, biodiversity and agricultural damage. The early-warning system for hantavirus risks should preferably be integrated in a general rodent monitoring system.
This monitoring system for rodents could be used to predict and facilitate recommendations for persons/animals in at-risk areas for a wide variety of diseases.
This monitoring system needs to be set up as a collaborative effort of Wageningen University and Research, Faunafonds, Zoogdiervereniging VZZ, CVI, Alterra and Cib/RIVM.

11. Control measures.

Hantaviruses are enveloped RNA viruses but unlike other enveloped RNA viruses, hantaviruses are quite stable in the environment. PUUV shows a prolonged survival outside the rodent host. PUUV remains infectious in bank vole cage beddings for 12-15 days at room temperature [128].
Hantaviruses are susceptible to UV-light, high temperatures, acidic and dry conditions.

Control measures are based on:
1) avoiding contact with rodents, their excreta and aerosolized virus particles,
2) decontamination.

Guidelines:

1. Prevent rodent colonization of the home and work environment by rodent control, reduction of available food sources and nesting opportunities (both inside and outside), prevention of rodent infestation of buildings.
2. Handle rodents (dead or alive), rodent excreta, rodent nests and rodent traps wearing plastic or rubber gloves. Soak gloves in disinfectant * *before* removing them. Thoroughly wash hands with soap and water upon removal of the gloves.
3. Turn your back against the wind when a) handling rodents, rodent excreta, rodent nests and rodent traps, b) digging earth in rodent infested areas, c) handling wood.
4. Avoid biting incidents when it is necessary to handle living rodents.
5. Avoid taking deep breaths while in close proximity to rodents, rodent excreta and rodent nests.

6. Place rodent carcasses in plastic bags containing sufficient disinfectant* to thoroughly soak the carcasses. Seal the bags before disposal.
7. Ventilate rodent infested indoor locations for at least 30 minutes to help remove any aerosolized virus inside the closed buildings *before* cleaning-up the location. Cleaning-up indoor locations: see points 2, 5, 6. Floors should be wet-mopped with water and disinfectant*. *No* vacuum cleaning or sweeping of dry surfaces before wet-mopping the floor.
8. Avoid exposure to rodents or rodent excreta while recreating (camping, hiking) in rodent infested areas.
9. Avoid exposure to rodent excreta by washing wild fruit before consumption.

   * Hantaviruses are lipid-enveloped viruses and therefore susceptible to a wide variety of detergents and alcohols. Effective disinfectants described in literature:

   Chlorine dioxide, 1-2%, > 10 min. (f.e. Clidox ®) [146]
   Chlorine, 10%, > 10-15 min. (f.e. Lysol®) [147]
   Chloroform [148]
   Ethanol (both absolute and 70%), > 30 min. [146,148,149]
   Hypochlorous acid 1-2%, >10 min. Virkon ® S [146]
   Methanol (absolute), > 10 min. [146,150]
   Methanol/acetone (1:1) > 10 min. [150]
   Paraformaldehyde 1%, >20 min. [150]
   Parachlorometaxylenol 1-5%, > 10 min. (Dettol®) [146]
   Peracetic acid 1%, > 10 min. [146]
   Phenol [148]
   ß-propiolactone [148]
   Sodium-P-toluene-sulfonchloramide 1-5%, > 10 min. (Halamid-d ®) [146]
   Sodium hypocloride 1%-10%, > 10 min. (general house-hold disinfectant: bleach) [146,148]
   UV-radiation > 3 min. [150]

   The choice of disinfectant depends on the situation in which it is used. For standard decontamination procedures in laboratories Clidox ®, Halamid-d® and Virkon® S are recommended [146]. For use in private house-holds the common house-hold disinfectant bleach is recommended.

These measures should be communicated to populations at risk through information leaflets as is done in Belgium, France and Germany as a reaction to the increased human incidence since 2005 [2]. These leaflets should be made available to the general public at general practitioners, camp grounds, leisure centers and tourist information offices in known hantavirus endemic regions and through the relevant health and safety executives ([arbodiensten]).

Rodent control in nature (f.e at hantavirus “hot spots”) is based on disturbance of the preferred habitats of the species that need to be controlled and the facilitation of the presence of their natural predators. Examples of such measures are removal of undergrowth, removal of masting trees, sowing of non-favourite grass

species, placement nestboxes. These measures will interfere deeply with excisting local ecosystems which is undesirable and illegal in the Netherlands based on the “Flora en Fauna wet” (see appendix 2).

Recommendation.

To draw up an information brochure about the risks of hantaviruses and the control measures that can be taken.

To arise public awareness by dispersal of information brochures to populations at risk f.e. through general practitioners, camp grounds, leisure centers and tourist information offices in known hantavirus endemic regions and through the relevant health and safety executives

Acknowledgements.
Paul Heyman, Joke van der Giessen, Marieta Braks, Johan Reimerink and Barbara Schimmer are acknowledged for critical reading of this report.

Literature.

1. Schneider F, Mossong J. Increased hantavirus infections in Luxembourg, August 2005. *Euro Surveill* 2005;10(8):E050825 1.
2. Mailles A, Sin MA, Ducoffre G, Heyman P, Koch J, Zeller H. Larger than usual increase in cases of hantavirus infections in Belgium, France and Germany, June 2005. *Euro Surveill* 2005;10(7):E050721 4.
3. Koch J, Brockmann SO, Winter C, Kimmig P, Stark K. Significant increase of hantavirus infections in Germany since the beginning of 2007. *Euro Surveill* 2007;12(5):E070503 1.
4. Heyman P, Vaheri A, members E. Situation of Hantavirus infections and Haemorrhagic fever with renal syndrome in European countries as of december 2006 *Eurosurveillance* 2008;13(28).
5. Koren N, Grilc E, Blasko M, Avsic T, Kraigher A. An increase in reported cases of haemorrhagic fever with renal syndrome in Slovenia in early 2008. *Euro Surveill* 2008;13(17).
6. Heyman P, Cochez C, Ducoffre G, et al. Haemorrhagic Fever with Renal Syndrome: an analysis of the outbreaks in Belgium, France, Germany, the Netherlands and Luxembourg in 2005. *Euro Surveill* 2007;12(5):E15-6.
7. Tersago K, Verhagen R, Servais A, Heyman P, Ducoffre G, Leirs H. Hantavirus disease (nephropathia epidemica) in Belgium: effects of tree seed production and climate. *Epidemiol Infect* 2008:1-7.
8. Clement J, Vercauteren J, Verstraeten WW, et al. Relating increasing hantavirus incidences to the changing climate: the mast connection. *Int J Health Geogr* 2009;8:1.
9. Plyusnin A, Morzunov SP. Virus evolution and genetic diversity of hantaviruses and their rodent hosts. *Curr Top Microbiol Immunol* 2001;256:47-75.
10. Asikainen K, Hanninen T, Henttonen H, et al. Molecular evolution of puumala hantavirus in Fennoscandia: phylogenetic analysis of strains from two recolonization routes, Karelia and Denmark. *J Gen Virol* 2000;81(Pt 12):2833-41.
11. Plyusnin A, Vapalahti O, Vaheri A. Hantaviruses: genome structure, expression and evolution. *J Gen Virol* 1996;77 (Pt 11):2677-87.
12. Meyer BJ, Schmaljohn CS. Persistent hantavirus infections: characteristics and mechanisms. *Trends Microbiol* 2000;8(2):61-7.
13. Carey DE, Reuben R, Panicker KN, Shope RE, Myers RM. Thottapalayam virus: a presumptive arbovirus isolated from a shrew in India. *Indian J Med Res* 1971;59(11):1758-60.
14. Zeller HG, Karabatsos N, Calisher CH, et al. Electron microscopic and antigenic studies of uncharacterized viruses. II. Evidence suggesting the placement of viruses in the family Bunyaviridae. *Arch Virol* 1989;108(3-4):211-27.
15. Klempa B, Fichet-Calvet E, Lecompte E, et al. Novel hantavirus sequences in Shrew, Guinea. *Emerg Infect Dis* 2007;13(3):520-2.
16. Song JW, Gu SH, Bennett SN, et al. Seewis virus, a genetically distinct hantavirus in the Eurasian common shrew (Sorex araneus). *Virol J* 2007;4:114.
17. Song JW, Kang HJ, Song KJ, et al. Newfound hantavirus in Chinese mole shrew, Vietnam. *Emerg Infect Dis* 2007;13(11):1784-7.
18. Arai S, Song JW, Sumibcay L, et al. Hantavirus in northern short-tailed shrew, United States. *Emerg Infect Dis* 2007;13(9):1420-3.
19. Arai S, Bennett SN, Sumibcay L, et al. Phylogenetically distinct hantaviruses in the masked shrew (Sorex cinereus) and dusky shrew (Sorex monticolus) in the United States. *Am J Trop Med Hyg* 2008;78(2):348-51.
20. Brummer-Korvenkontio M, Vaheri A, Hovi T, et al. Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. *J Infect Dis* 1980;141(2):131-4.

21. Plyusnin A, Vapalahti O, Lankinen H, et al. Tula virus: a newly detected hantavirus carried by European common voles. *J Virol* 1994;68(12):7833-9.
22. Lee PW, Svedmyr A, Amyx HL, et al. HFRS antigen and antibody in two species of Swedish voles. *Scand J Infect Dis* 1982;14(4):315-6.
23. Hjelle B, Krolikowski J, Torrez-Martinez N, Chavez-Giles F, Vanner C, Laposata E. Phylogenetically distinct hantavirus implicated in a case of hantavirus pulmonary syndrome in the northeastern United States. *J Med Virol* 1995;46(1):21-7.
24. Song W, Torrez-Martinez N, Irwin W, et al. Isla Vista virus: a genetically novel hantavirus of the California vole Microtus californicus. *J Gen Virol* 1995;76 (Pt 12):3195-9.
25. Horling J, Chizhikov V, Lundkvist A, et al. Khabarovsk virus: a phylogenetically and serologically distinct hantavirus isolated from Microtus fortis trapped in far-east Russia. *J Gen Virol* 1996;77 (Pt 4):687-94.
26. Plyusnin A, Vapalahti O, Lundkvist A, Henttonen H, Vaheri A. Newly recognised hantavirus in Siberian lemmings. *Lancet* 1996;347(9018):1835.
27. Kariwa H, Yoshimatsu K, Araki K, et al. Detection of hantaviral antibodies among patients with hepatitis of unknown etiology in Japan. *Microbiol Immunol* 2000;44(5):357-62.
28. Song KJ, Baek LJ, Moon S, et al. Muju virus, a novel hantavirus harboured by the arvicolid rodent Myodes regulus in Korea. *J Gen Virol* 2007;88(Pt 11):3121-9.
29. Avsic-Zupanc T, Xiao SY, Stojanovic R, Gligic A, van der Groen G, LeDuc JW. Characterization of Dobrava virus: a Hantavirus from Slovenia, Yugoslavia. *J Med Virol* 1992;38(2):132-7.
30. Plyusnin A, Kruger DH, Lundkvist A. Hantavirus infections in Europe. *Adv Virus Res* 2001;57:105-36.
31. Lee HW, Baek LJ, Johnson KM. Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic fever, from wild urban rats. *J Infect Dis* 1982;146(5):638-44.
32. Lee HW, Lee PW, Johnson KM. Isolation of the etiologic agent of Korean hemorrhagic fever. 1978. *J Infect Dis* 2004;190(9):1711-21.
33. Klempa B, Fichet-Calvet E, Lecompte E, et al. Hantavirus in African wood mouse, Guinea. *Emerg Infect Dis* 2006;12(5):838-40.
34. Baek LJ, Kariwa H, Lokugamage K, et al. Soochong virus: an antigenically and genetically distinct hantavirus isolated from Apodemus peninsulae in Korea. *J Med Virol* 2006;78(2):290-7.
35. Elwell MR, Ward GS, Tingpalapong M, LeDuc JW. Serologic evidence of Hantaan-like virus in rodents and man in Thailand. *Southeast Asian J Trop Med Public Health* 1985;16(3):349-54.
36. Yashina L, Mishin V, Zdanovskaya N, Schmaljohn C, Ivanov L. A newly discovered variant of a hantavirus in Apodemus peninsulae, far Eastern Russia. *Emerg Infect Dis* 2001;7(5):912-3.
37. Nichol ST, Spiropoulou CF, Morzunov S, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science* 1993;262(5135):914-7.
38. Song JW, Baek LJ, Gajdusek DC, et al. Isolation of pathogenic hantavirus from white-footed mouse (Peromyscus leucopus). *Lancet* 1994;344(8937):1637.
39. Rollin PE, Ksiazek TG, Elliott LH, et al. Isolation of black creek canal virus, a new hantavirus from Sigmodon hispidus in Florida. *J Med Virol* 1995;46(1):35-9.
40. Morzunov SP, Feldmann H, Spiropoulou CF, et al. A newly recognized virus associated with a fatal case of hantavirus pulmonary syndrome in Louisiana. *J Virol* 1995;69(3):1980-3.
41. Rawlings JA, Torrez-Martinez N, Neill SU, et al. Cocirculation of multiple hantaviruses in Texas, with characterization of the small (S) genome of a previously undescribed virus of cotton rats (Sigmodon hispidus). *Am J Trop Med Hyg* 1996;55(6):672-9.
42. Song JW, Baek LJ, Nagle JW, Schlitter D, Yanagihara R. Genetic and phylogenetic analyses of hantaviral sequences amplified from archival tissues of deer mice

(Peromyscus maniculatus nubiterrae) captured in the eastern United States. *Arch Virol* 1996;141(5):959-67.

43. Sanchez AJ, Abbott KD, Nichol ST. Genetic identification and characterization of limestone canyon virus, a unique Peromyscus-borne hantavirus. *Virology* 2001;286(2):345-53.

44. Morzunov SP, Rowe JE, Ksiazek TG, Peters CJ, St Jeor SC, Nichol ST. Genetic analysis of the diversity and origin of hantaviruses in Peromyscus leucopus mice in North America. *J Virol* 1998;72(1):57-64.

45. Hjelle B, Chavez-Giles F, Torrez-Martinez N, et al. Genetic identification of a novel hantavirus of the harvest mouse Reithrodontomys megalotis. *J Virol* 1994;68(10):6751-4.

46. Hjelle B, Anderson B, Torrez-Martinez N, Song W, Gannon WL, Yates TL. Prevalence and geographic genetic variation of hantaviruses of New World harvest mice (Reithrodontomys): identification of a divergent genotype from a Costa Rican Reithrodontomys mexicanus. *Virology* 1995;207(2):452-9.

47. Fulhorst CF, Monroe MC, Salas RA, et al. Isolation, characterization and geographic distribution of Cano Delgadito virus, a newly discovered South American hantavirus (family Bunyaviridae). *Virus Res* 1997;51(2):159-71.

48. Johnson AM, de Souza LT, Ferreira IB, et al. Genetic investigation of novel hantaviruses causing fatal HPS in Brazil. *J Med Virol* 1999;59(4):527-35.

49. Bharadwaj M, Botten J, Torrez-Martinez N, Hjelle B. Rio Mamore virus: genetic characterization of a newly recognized hantavirus of the pygmy rice rat, Oligoryzomys microtis, from Bolivia. *Am J Trop Med Hyg* 1997;57(3):368-74.

50. Johnson AM, Bowen MD, Ksiazek TG, et al. Laguna Negra virus associated with HPS in western Paraguay and Bolivia. *Virology* 1997;238(1):115-27.

51. Lopez N, Padula P, Rossi C, et al. Genetic characterization and phylogeny of Andes virus and variants from Argentina and Chile. *Virus Res* 1997;50(1):77-84.

52. Levis S, Morzunov SP, Rowe JE, et al. Genetic diversity and epidemiology of hantaviruses in Argentina. *J Infect Dis* 1998;177(3):529-38.

53. Levis S, Rowe JE, Morzunov S, Enria DA, St Jeor S. New hantaviruses causing hantavirus pulmonary syndrome in central Argentina. *Lancet* 1997;349(9057):998-9.

54. Vincent MJ, Quiroz E, Gracia F, et al. Hantavirus pulmonary syndrome in Panama: identification of novel hantaviruses and their likely reservoirs. *Virology* 2000;277(1):14-9.

55. Fulhorst CF, Cajimat MN, Utrera A, Milazzo ML, Duno GM. Maporal virus, a hantavirus associated with the fulvous pygmy rice rat (Oligoryzomys fulvescens) in western Venezuela. *Virus Res* 2004;104(2):139-44.

56. Klingstrom J, Heyman P, Escutenaire S, et al. Rodent host specificity of European hantaviruses: evidence of Puumala virus interspecific spillover. *J Med Virol* 2002;68(4):581-8.

57. Sauvage F, Penalba C, Vuillaume P, et al. Puumala hantavirus infection in humans and in the reservoir host, Ardennes region, France. *Emerg Infect Dis* 2002;8(12):1509-11.

58. Vahlenkamp M, Muller T, Tackmann K, Loschner U, Schmitz H, Schreiber M. The muskrat (Ondatra zibethicus) as a new reservoir for puumala-like hantavirus strains in Europe. *Virus Res* 1998;57(2):139-50.

59. Scharninghausen JJ, Pfeffer M, Meyer H, Davis DS, Honeycutt RL, Faulde M. Genetic evidence for tula virus in Microtus arvalis and Microtus agrestis populations in Croatia. *Vector Borne Zoonotic Dis* 2002;2(1):19-27.

60. Song JW, Gligic A, Yanagihara R. Identification of Tula hantavirus in Pitymys subterraneus captured in the Cacak region of Serbia-Yugoslavia. *Int J Infect Dis* 2002;6(1):31-6.

61. Padula PJ, Edelstein A, Miguel SD, Lopez NM, Rossi CM, Rabinovich RD. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. *Virology* 1998;241(2):323-30.

62. Gavrilovskaya IN, Apekina NS, Myasnikov Yu A, et al. Features of circulation of hemorrhagic fever with renal syndrome (HFRS) virus among small mammals in the European U.S.S.R. *Arch Virol* 1983;75(4):313-6.
63. Lee HW, van der Groen G. Hemorrhagic fever with renal syndrome. *Prog Med Virol* 1989;36:62-102.
64. Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. *Emerg Infect Dis* 1997;3(2):95-104.
65. Tkachenko EA, Ivanov AP, Donets MA, et al. Potential reservoir and vectors of haemorrhagic fever with renal syndrome (HFRS) in the U. S. S. R. *Ann Soc Belg Med Trop* 1983;63(3):267-9.
66. Groen J, Gerding MN, Jordans JG, Clement JP, Nieuwenhuijs JH, Osterhaus AD. Hantavirus infections in The Netherlands: epidemiology and disease. *Epidemiol Infect* 1995;114(2):373-83.
67. Mills JN. Regulation of rodent-borne viruses in the natural host: implications for human disease. *Arch Virol Suppl* 2005(19):45-57.
68. Jensen TS. Seed production and outbreaks of non-cyclic rodent populations in deciduous forests. *Oecologia* 1982;54(2):184-192.
69. Crespin L, Verhagen R, Stenseth NC, Yoccoz NG, Pre?vot-Julliard AC, Lebreton JD. Survival in fluctuating bank vole populations: Seasonal and yearly variations. *Oikos* 2002;98(3):467-479.
70. Vapalahti O, Mustonen J, Lundkvist A, Henttonen H, Plyusnin A, Vaheri A. Hantavirus infections in Europe. *Lancet Infect Dis* 2003;3(10):653-61.
71. Kramski M, Achazi K, Klempa B, Kruger DH. Nephropathia epidemica with a 6-week incubation period after occupational exposure to Puumala hantavirus. *J Clin Virol* 2009;44(1):99-101.
72. Brummer-Korvenkontio M, Vapalahti O, Henttonen H, Koskela P, Kuusisto P, Vaheri A. Epidemiological study of nephropathia epidemica in Finland 1989-96. *Scand J Infect Dis* 1999;31(5):427-35.
73. Rollin PE, Coudrier D, Sureau P. Hantavirus epidemic in Europe, 1993. *Lancet* 1994;343(8889):115-6.
74. Forslund T, Saltevo J, Anttinen J, et al. Complications of nephropathia epidemica: three cases. *J Intern Med* 1992;232(1):87-90.
75. Launes J, Hautanen A. Nephropathia epidemica encephalitis. *Acta Neurol Scand* 1988;78(3):234-5.
76. Esselink RA, Gerding MN, Brouwers PJ, et al. Guillain-Barre syndrome associated with hantavirus infection. *Lancet* 1994;343(8890):180-1.
77. Toivanen AL, Valanne L, Tatlisumak T. Acute disseminated encephalomyelitis following nephropathia epidemica. *Acta Neurol Scand* 2002;105(4):333-6.
78. Ahlm C, Settergren B, Gothefors L, Juto P. Nephropathia epidemica (hemorrhagic fever with renal syndrome) in children: clinical characteristics. *Pediatr Infect Dis J* 1994;13(1):45-9.
79. Mustonen J, Huttunen NP, Brummer-Korvenkontio M, Vaheri A. Clinical picture of nephropathia epidemica in children. *Acta Paediatr* 1994;83(5):526-9.
80. Schultze D, Lundkvist A, Blauenstein U, Heyman P. Tula virus infection associated with fever and exanthema after a wild rodent bite. *Eur J Clin Microbiol Infect Dis* 2002;21(4):304-6.
81. Klempa B, Meisel H, Rath S, Bartel J, Ulrich R, Kruger DH. Occurrence of renal and pulmonary syndrome in a region of northeast Germany where Tula hantavirus circulates. *J Clin Microbiol* 2003;41(10):4894-7.
82. Maes P, Clement J, Gavrilovskaya I, Van Ranst M. Hantaviruses: immunology, treatment, and prevention. *Viral Immunol* 2004;17(4):481-97.
83. Hjelle B. Vaccines against hantaviruses. *Expert Rev Vaccines* 2002;1(3):373-84.
84. Lundkvist A, Bjorsten S, Niklasson B. Immunoglobulin G subclass responses against the structural components of Puumala virus. *J Clin Microbiol* 1993;31(2):368-72.

85. Maes P, Clement J, Van Ranst M. Recent approaches in hantavirus vaccine development. *Expert Rev Vaccines* 2009;8(1):67-76.
86. Nemirov K, Andersen HK, Leirs H, et al. Saaremaa hantavirus in Denmark. *J Clin Virol* 2004;30(3):254-7.
87. Kallio ER, Voutilainen L, Vapalahti O, et al. Endemic hantavirus infection impairs the winter survival of its rodent host. *Ecology* 2007;88(8):1911-6.
88. Netski D, Thran BH, St Jeor SC. Sin Nombre virus pathogenesis in Peromyscus maniculatus. *J Virol* 1999;73(1):585-91.
89. Lyubsky S, Gavrilovskaya I, Luft B, Mackow E. Histopathology of Peromyscus leucopus naturally infected with pathogenic NY-1 hantaviruses: pathologic markers of HPS viral infection in mice. *Lab Invest* 1996;74(3):627-33.
90. Compton SR, Jacoby RO, Paturzo FX, Smith AL. Persistent Seoul virus infection in Lewis rats. *Arch Virol* 2004;149(7):1325-39.
91. Plyusnin A, Horling J, Kanerva M, et al. Puumala hantavirus genome in patients with nephropathia epidemica: correlation of PCR positivity with HLA haplotype and link to viral sequences in local rodents. *J Clin Microbiol* 1997;35(5):1090-6.
92. Elgh F, Linderholm M, Wadell G, Tarnvik A, Juto P. Development of humoral cross-reactivity to the nucleocapsid protein of heterologous hantaviruses in nephropathia epidemica. *FEMS Immunol Med Microbiol* 1998;22(4):309-15.
93. Kallio-Kokko H, Vapalahti O, Lundkvist A, Vaheri A. Evaluation of Puumala virus IgG and IgM enzyme immunoassays based on recombinant baculovirus-expressed nucleocapsid protein for early nephropathia epidemica diagnosis. *Clin Diagn Virol* 1998;10(1):83-90.
94. Kallio-Kokko H, Leveelahti R, Brummer-Korvenkontio M, Lundkvist A, Vaheri A, Vapalahti O. Human immune response to Puumala virus glycoproteins and nucleocapsid protein expressed in mammalian cells. *J Med Virol* 2001;65(3):605-13.
95. Vapalahti O, Kallio-Kokko H, Narvanen A, et al. Human B-cell epitopes of Puumala virus nucleocapsid protein, the major antigen in early serological response. *J Med Virol* 1995;46(4):293-303.
96. Elgh F, Lundkvist A, Alexeyev OA, et al. Serological diagnosis of hantavirus infections by an enzyme-linked immunosorbent assay based on detection of immunoglobulin G and M responses to recombinant nucleocapsid proteins of five viral serotypes. *J Clin Microbiol* 1997;35(5):1122-30.
97. Lundkvist A, Hukic M, Horling J, Gilljam M, Nichol S, Niklasson B. Puumala and Dobrava viruses cause hemorrhagic fever with renal syndrome in Bosnia-Herzegovina: evidence of highly cross-neutralizing antibody responses in early patient sera. *J Med Virol* 1997;53(1):51-9.
98. Pettersson L, Boman J, Juto P, Evander M, Ahlm C. Outbreak of Puumala virus infection, Sweden. *Emerg Infect Dis* 2008;14(5):808-10.
99. Hofmann J, Meisel H, Klempa B, et al. Hantavirus outbreak, Germany, 2007. *Emerg Infect Dis* 2008;14(5):850-2.
100. Abu Sin M, Stark K, van Treeck U, et al. Risk factors for hantavirus infection in Germany, 2005. *Emerg Infect Dis* 2007;13(9):1364-6.
101. Schimmer B, van Oudheusden D, Reimerink J, Swaan CM, Reusken C. Een cluster van hantavirusinfecties in het zuidoosten van Noord-Brabant. *Infectieziektenbulletin* 2008;19(4):138-142.
102. Klein SL, Calisher CH. Emergence and persistence of hantaviruses. *Curr Top Microbiol Immunol* 2007;315:217-52.
103. Mills JN, Ksiazek TG, Ellis BA, et al. Patterns of association with host and habitat: antibody reactive with Sin Nombre virus in small mammals in the major biotic communities of the southwestern United States. *Am J Trop Med Hyg* 1997;56(3):273-84.
104. Kallio-Kokko H, Uzcategui N, Vapalahti O, Vaheri A. Viral zoonoses in Europe. *FEMS Microbiol Rev* 2005;29(5):1051-77.

105. Heyman P, Klingstrom J, de Jaegere F, et al. Tula hantavirus in Belgium. *Epidemiol Infect* 2002;128(2):251-6.
106. Plyusnina A, Deter J, Charbonnel N, Cosson JF, Plyusnin A. Puumala and Tula hantaviruses in France. *Virus Res* 2007;129(1):58-63.
107. Bowen MD, Gelbmann W, Ksiazek TG, Nichol ST, Nowotny N. Puumala virus and two genetic variants of Tula virus are present in Austrian rodents. *J Med Virol* 1997;53(2):174-81.
108. Heyman P, Baert K, Plyusnina A, et al. Serological and genetic evidence for the presence of Seoul hantavirus in Rattus norvegicus in Flanders, Belgium. *Scand J Infect Dis* 2008:1-6.
109. Heyman P, Plyusnina A, Berny P, et al. Seoul hantavirus in Europe: first demonstration of the virus genome in wild Rattus norvegicus captured in France. *Eur J Clin Microbiol Infect Dis* 2004;23(9):711-7.
110. Sauvage F, Langlais M, Pontier D. Predicting the emergence of human hantavirus disease using a combination of viral dynamics and rodent demographic patterns. *Epidemiol Infect* 2007;135(1):46-56.
111. Escutenaire S, Chalon P, Verhagen R, et al. Spatial and temporal dynamics of Puumala hantavirus infection in red bank vole (Clethrionomys glareolus) populations in Belgium. *Virus Res* 2000;67(1):91-107.
112. Escutenaire S, Chalon P, De Jaegere F, et al. Behavioral, physiologic, and habitat influences on the dynamics of Puumala virus infection in bank voles (Clethrionomys glareolus). *Emerg Infect Dis* 2002;8(9):930-6.
113. Wolf C, Langlais M, Sauvage F, Pontier D. A multi-patch epidemic model with periodic demography, direct and indirect transmission and variable maturation rate. *Mathematical Population Studies* 2006;13(3):153-177.
114. Jordans JG, Groen J, Clement J, Lefevre A, Haraldsdottir V, Osterhaus AD. [Hantaan virus infection in Twente]. *Ned Tijdschr Geneeskd* 1991;135(18):796-8.
115. Dillingh SJ, Jira P, Morroy G, Wolters B, Beutler J, Schneeberger PM. [Two patients with Hantavirus infection in The Netherlands; substantial increase in incidence in neighbouring countries]. *Ned Tijdschr Geneeskd* 2006;150(23):1303-6.
116. Reusken C, de Vries A, Adema J, et al. First genetic detection of Tula hantavirus in wild rodents in the Netherlands. *J Infect* 2008;57(6):500-3.
117. Heyman P, Van Mele R, De Jaegere F, et al. Distribution of hantavirus foci in Belgium. *Acta Trop* 2002;84(3):183-8.
118. Hardestam J, Karlsson M, Falk KI, Olsson G, Klingstrom J, Lundkvist A. Puumala hantavirus excretion kinetics in bank voles (Myodes glareolus). *Emerg Infect Dis* 2008;14(8):1209-15.
119. Glass GE, Childs JE, Korch GW, LeDuc JW. Association of intraspecific wounding with hantaviral infection in wild rats (Rattus norvegicus). *Epidemiol Infect* 1988;101(2):459-72.
120. Hinson ER, Shone SM, Zink MC, Glass GE, Klein SL. Wounding: the primary mode of Seoul virus transmission among male Norway rats. *Am J Trop Med Hyg* 2004;70(3):310-7.
121. Kariwa H, Fujiki M, Yoshimatsu K, Arikawa J, Takashima I, Hashimoto N. Urine-associated horizontal transmission of Seoul virus among rats. *Arch Virol* 1998;143(1):15-24.
122. Botten J, Mirowsky K, Ye C, et al. Shedding and intracage transmission of Sin Nombre hantavirus in the deer mouse (Peromyscus maniculatus) model. *J Virol* 2002;76(15):7587-94.
123. Bernshtein AD, Apekina NS, Mikhailova TV, et al. Dynamics of Puumala hantavirus infection in naturally infected bank voles (Clethrinomys glareolus). *Arch Virol* 1999;144(12):2415-28.
124. Niklasson B, Hornfeldt B, Lundkvist A, Bjorsten S, Leduc J. Temporal dynamics of Puumala virus antibody prevalence in voles and of nephropathia epidemica incidence in humans. *Am J Trop Med Hyg* 1995;53(2):134-40.

125. Yanagihara R, Svedmyr A, Amyx HL, et al. Isolation and propagation of nephropathia epidemica virus in bank voles. *Scand J Infect Dis* 1984;16(3):225-8.
126. Sauvage F, Langlais M, Yoccoz NG, Pontier D. Modelling hantavirus in cyclic bank voles: the role of indirect transmission on virus persistence. *Journal of Animal Ecology* 2003;72:1-13.
127. Olsson GE, White N, Ahlm C, et al. Demographic factors associated with hantavirus infection in bank voles (Clethrionomys glareolus). *Emerg Infect Dis* 2002;8(9):924-9.
128. Kallio ER, Klingstrom J, Gustafsson E, et al. Prolonged survival of Puumala hantavirus outside the host: evidence for indirect transmission via the environment. *J Gen Virol* 2006;87(Pt 8):2127-34.
129. Verhagen R, Leirs H, Tkachenko E, van der Groen G. Ecological and epidemiological data on Hantavirus in bank vole populations in Belgium. *Arch Virol* 1986;91(3-4):193-205.
130. Linard C, Lamarque P, Heyman P, et al. Determinants of the geographic distribution of Puumala virus and Lyme borreliosis infections in Belgium. *Int J Health Geogr* 2007;6:15.
131. Vapalahti O, Lundkvist A, Fedorov V, et al. Isolation and characterization of a hantavirus from Lemmus sibiricus: evidence for host switch during hantavirus evolution. *J Virol* 1999;73(7):5586-92.
132. Ims RA. Responses in spatial organization and behaviour to manipulations of the food resource in the vole Clethrionomys rufocanus. *Journal of Animal Ecology* 1987;56(2):585-596.
133. Lofgren O. Spatial organization of cyclic Clethrionomys females: Occupancy of all available space at peak densities? *Oikos* 1995;72(1):29-35.
134. Deter J, Chaval Y, Galan M, et al. Kinship, dispersal and hantavirus transmission in bank and common voles. *Arch Virol* 2008;153(3):435-44.
135. Dobly A. Movement patterns of male common voles (Microtus arvalis) in a network of Y junctions: Role of distant visual cues and scent marks. *Canadian Journal of Zoology* 2001;79(12):2228-2238.
136. Linard C, Tersago K, Leirs H, Lambin EF. Environmental conditions and Puumala virus transmission in Belgium. *Int J Health Geogr* 2007;6:55.
137. Nystrom K. Incidence and prevalence of endemic benign (epidemic) nephropathy in AC county, Sweden, in relation to population density and prevalence of small rodents. *Acta Med Scand Suppl* 1977;609:1-92.
138. Olsson GE, Dalerum F, Hornfeldt B, et al. Human hantavirus infections, Sweden. *Emerg Infect Dis* 2003;9(11):1395-401.
139. Olsson GE, Hjertqvist M, Lundkvist A, Hornfeldt B. Predicting high risk for human hantavirus infections, Sweden. *Emerg Infect Dis* 2009;15(1):104-6.
140. Hansson L. Clethrionomys food: generic, specific and regional characteristics. *Annales Zoologici Fennici* 1985;22(3):315-318.
141. Van Loock F, Thomas I, Clement J, Ghoos S, Colson P. A case-control study after a hantavirus infection outbreak in the south of Belgium: who is at risk? *Clin Infect Dis* 1999;28(4):834-9.
142. Penalba C, Galempoix JM, Lanoux P. Epidemiology of hemorrhagic fever with renal syndrome in france. *E?pide?miologie des infections a? hantavirus en france* 2001;31(SUPPL. 2):272-284.
143. Crowcroft NS, Infuso A, Ilef D, et al. Risk factors for human hantavirus infection: Franco-Belgian collaborative case-control study during 1995-6 epidemic. *Bmj* 1999;318(7200):1737-8.
144. Winter CH, Brockmann SO, Piechotowski I, et al. Survey and case-control study during epidemics of Puumala virus infection. *Epidemiol Infect* 2009:1-7.
145. Heyman P, Vaheri A, Lundkvist A, Avsic-Zupanc T. Hantavirus infections in Europe: from virus carriers to a major public-health problem. *Expert Rev Anti Infect Ther* 2009;7(2):205-17.

146. Maes P, Li S, Verbeeck J, Keyaerts E, Clement J, Van Ranst M. Evaluation of the efficacy of disinfectants against Puumala hantavirus by real-time RT-PCR. ***J Virol Methods*** **2007;141(1):111-5.**

147. Fink TM. Rodents, human remains, and North American hantaviruses: risk factors and prevention measures for forensic science personnel--a review. ***J Forensic Sci*** **1996;41(6):1052-6.**

148. Schmaljohn C, Huggins J, Calisher CH. Laboratory and field safety. ***In: Lee, H.W., Calisher, C., Schmaljohn, C. (eds.) Manual of Hemorrhagic Fever with Renal Syndrome and Hantavirus Pulmonary Syndrome. WHO Collaborating Center for Virus Reference and Research (Hantaviruses), Asan Institute for Life Sciences, Seoul.*** **1999:191-198.**

149. Hardestam J, Simon M, Hedlund KO, Vaheri A, Klingstrom J, Lundkvist A. Ex vivo stability of the rodent-borne Hantaan virus in comparison to that of arthropod-borne members of the Bunyaviridae family. ***Appl Environ Microbiol*** **2007;73(8):2547-51.**

150. Kraus AA, Priemer C, Heider H, Kruger DH, Ulrich R. Inactivation of Hantaan virus-containing samples for subsequent investigations outside biosafety level 3 facilities. ***Intervirology*** **2005;48(4):255-61.**

151. Hansson L, Je?drzejewska B, Je?drzejewski W. Regional differences in dynamics of bank vole populations in europe. ***Polish Journal of Ecology*** **2000;48(SUPPL.):163-177.**

152. Verhagen R, Leirs H, Verheyen W. Demography of Clethrionomys glareolus in Belgium. ***Polish Journal of Ecology*** **2000;48(SUPPL.):113-123.**

153. Gliwicz J, Ims RA. Dispersal in the bank vole. ***Polish Journal of Ecology*** **2000;48(SUPPL.):51-61.**

Appendix 1. Characteristics of bank vole population structure and dynamics relevant for hantavirus epidemiology.

1. short life expectancy of several months.
2. communal nesting as a common overwintering strategy.
3. specific habitat mainly composed of broadleaf forest (beech, oak) with dense undergrowth. In western and central Europe the highest bank vole densities are observed predominantly within broadleaf and mixed coniferous forests [151] [152].
4. habitat quality correlates with density of shelters and plant cover rather than specific sources of food. Abundance and spatial distribution of shelters/plant cover determine local bank vole densities which may therefore differ substantially within a forest area.
5. smaller bank vole territories in high quality habitats.
6. territories are delimited through urine deposit by the owner; supposedly on a daily basis. Urine marks will be explored by other specimens. Dominant and subordinate voles will deposit a differential pattern of urine marks.
7. abundance and spatial distribution of shelters determine the number of suitable places for sexually active females who are territorial when breeding.
8. bank voles rarely leave forests.
9. Intra-and interannual rapid fluctuating population densities. These changes are a result of a changing reproductive output ; between 1 up to 4 generations can follow each other in the summer of a peak year.
10. litter size and length of reproductive season vary among years.
11. the amount of offspring is related to number of breeding females. The number of breeding females changes with respect to population densities; at high densities the voles mature later and at a lower rate than at low population densities.
12. juveniles disperse to find their own territory to reproduce, usually within the first 2-3 months upon birth.
13. bank voles have considerable dispersal propensities. Distances covered can reach more than 2-3 km in homogeneous landscapes (Henttonen*, pers. comm*.) and are estimated to be up to 500 meters in patchy landscapes [153].
14. pressure to disperse is lower in high quality habitats: more juveniles stay and settle in their natal patch.
15. sexual maturity follows dispersal of juveniles as soon as a new territory has been inhabitated .
16. bank vole population densities are strongly influenced by climatic conditions which control vegetation growth, snow cover and food supply, f.e. mast production [7,8].

Add. 16. Fluctuations in rodent densities in north-western Europe (temperate broadleaf forest biome) are almost solely related to seed production by broadleaf forests (beech, oak). In Nordic countries fluctuations in rodent densities are predator-driven (taiga biome with boreal forest and hardly any broadleaf seed production). Peaks in bank vole densities in north-western Europe correlate to peaks in seed production (mast years) [68] [69]. An abundance of resources can a) improve winter survival, b) elongate the breeding period, c) result in a higher proportion of breeding females and d) induce winter breeding ([7] and reference therein). As a consequence the bank vole population densities will remain high from autumn until next spring. Factors influencing tree seed production will indirectly influence bank vole densities and therefore ultimately the human PUUV incidence.

Appendix 2. Extract Flora- en Fauna wet.

“Onder de Flora- en faunawet zijn als beschermde soort aangewezen alle van nature in Nederland voorkomende zoogdierensoorten behalve de zwarte rat (*Rattus rattus*) , de bruine rat (*Rattus norvegicus*) en de huismuis (*Mus musculus*).”

Paragraaf 2. Bepalingen betreffende dieren in hun natuurlijke leefomgeving

Artikel 9

Het is verboden dieren, behorende tot een beschermde inheemse diersoort, te doden, te verwonden, te vangen, te bemachtigen of met het oog daarop op te sporen.

Artikel 10

Het is verboden dieren, behorende tot een beschermde inheemse diersoort, opzettelijk te verontrusten.

Artikel 11

Het is verboden nesten, holen of andere voortplantings- of vaste rust- of verblijfplaatsen van dieren, behorende tot een beschermde inheemse diersoort, te beschadigen, te vernielen, uit te halen, weg te nemen of te verstoren.

Taken from (april 6 2009):
http://www.minlnv.nl/portal/page?_pageid=116,1640898&_dad=portal&_schema=PORTAL&p_document_id=110637&p_node_id=1964090&p_mode=BROWSE

RIVM

National Institute
for Public Health
and the Environment

P.O. Box 1
3720 BA Bilthoven
The Netherlands
www.rivm.com