Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

SPATIAL AND TEMPORAL DYNAMICS OF PUUMALA HANTAVIRUS INFECTION IN RED BANK VOLE (*CLETHRIONOMYS GLAREOLUS*) POPULATIONS IN BELGIUM

S. Escutenaire [a], P. Chalon [a], R. Verhagen [b], P. Heyman [c], I. Thomas [d], L. Karelle-Bui [a], T. Avsic-Zupanc [e], A. Lundkvist [f], A. Plyusnin [g], P.-P. Pastoret [a]

[a] *Department of Immunology-Vaccinology, Faculty of Veterinary Medicine, University of Liège, Bât B 43 bis, Boulevard de Colonster, 20, 4000 Liège, Belgium*
[b] *Department of Evolutionary Biology, RUCA, 2020 Antwerp, Belgium*
[c] *Research Laboratory for Vector-Borne Diseases, Queen Astrid Military Hospital, 1120 Brussels, Belgium*
[d] *Louis Pasteur Public Health Institute, 1050 Brussels, Belgium*
[e] *Institute of Microbiology, Medical Faculty of Ljubljana, Ljubljana, Slovenia*
[f] *Swedish Institute for Infectious Disease Control, and Microbiology and Tumour Biology Centre, Stockholm, Sweden*
[g] *Department of Virology, Haartman Institute, University of Helsinki, FIN-00014 Helsinki, Finland*

KEYWORDS: Hantavirus; Puumala virus; *Clethrionomys glareolus*; Belgium

ABSTRACT

Dynamics of hantavirus infection and population densities in rodents were investigated from 1996 to 1999 in southern Belgium. Evidence of Puumala infection was restricted to *Clethrionomys glareolus*. Although the serotype was not determined, antibodies against hantavirus were also found in eight *Apodemus sylvaticus*. In fall 1996, the seroprevalence in C. glareolus was high (20.1%, 37 of 184) and the infection was widely distributed in the area studied whereas a focal occurrence of positive rodents and lower seroprevalence rates were recorded in spring 1997 (14.3%, six of 42), fall 1997 (6.6%, 11 of 166), spring 1998 (6.4%, three of 47) and fall 1998 (6.7%, 11 of 165). A pullulation of rodents was observed in spring 1999 and was associated with a markedly higher seroprevalence in C. *glareolus* (47.7%, 189 of 396). In all seasons, infection rates in adults were higher than in juveniles and subadults. No significant difference of prevalence was recorded between males and females. In two trapping sites, the temporary disappearance of positive animals after a crash in rodent populations suggests that a threshold in density is necessary for the maintenance of the enzootic cycle.

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Introduction

Hantaviruses are the etiological agents of zoonotic diseases called haemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and hantavirus pulmonary syndrome (HPS) in the Americas (Gajdusek, 1962; World Health Organization, 1983; Duchin et al., 1994). Hantaviruses constitute a group of antigenically and genetically related negative-stranded RNA viruses in the family *Bunyaviridae* (Schmaljohn and Dalrymple, 1983; Schmaljohn et al., 1985). Their genome consists of a small (S), medium (M) and large (L) segment encoding, respectively, a nucleocapsid protein (N), two glycoproteins G1 and G2 and an RNA polymerase (Elliott, 1990; Antic et al., 1992). Each hantavirus is associated predominantly with one host rodent or insectivore species (for reviews see Plyusnin et al., 1996; Schmaljohn and Hjelle, 1997). Sin Nombre (SN) and related hantaviruses causing HPS in the Americas are carried by *Sigmodontinae* rodents (Nichol et al., 1993; Schmaljohn and Hjelle, 1997). The case-fatality ratio averages 45% (Young et al., 1998). The striped field mouse (*Apodemus agrarius*) is the natural host of Hantaan virus (HTN) in Asia (Lee et al., 1978). The yellow-necked field mouse (*Apodemus flavicollis*) harbours Dobrava virus (DOB) in the Balkans (Avsic-Zupanc et al., 1992). DOB virus was also reported in Russia (Lundkvist et al., 1997a) and Estonia (Plyusnin et al., 1997; Lundkvist et al., 1998) where viral genome sequences were detected in A. *agrarius*. Both HTN and DOB viruses are associated with severe human disease (Lee et al., 1978; Avsic-Zupanc et al., 1999). Seoul virus (SEO) infection in humans usually has a milder clinical course and is spread by urban rats (*Rattus rattus* and *Rattus norvegicus*) (LeDuc et al., 1982; Lee et al., 1982). Detection of antibodies in rats from major harbours around the world seems to indicate a widespread distribution of SEO virus (for review see Mertz et al., 1998). The milder form of HFRS observed in western and northern Europe is caused by Puumala virus (PUU) carried by the red bank vole (*Clethrionomys glareolus*) (Brummer-Korvenkontio et al., 1980). Transmission of hantaviruses occurs through prolonged shedding in saliva, urine and faeces (Yanagihara et al., 1985; Tsai, 1987; Gavrilovskaya et al., 1990). Exposure to nesting materials, grooming behaviour and intraspecies wounding by bite are important for the maintenance of the enzootic cycle (Glass et al., 1988; Yanagihara and Gajdusek, 1988). Hantavirus infection persists in the host species apparently without clinical signs (Yanagihara et al., 1985; Childs et al., 1994). However, recent findings showed tissue pathology features in *Peromyscus maniculatus* and *Peromyscus leucopus* infected with SN and New York viruses, respectively (Lyubsky et al., 1996; Netski et al., 1999). Outbreaks of hantaviral disease generally occur following irruption of reservoir populations (LeDuc, 1987; Niklasson and LeDuc, 1987; Parmenter and Vigil, 1993). In northern Europe, the highest incidence of human cases coincides with the years when cyclic populations of red bank voles reach a peak (Nystrom, 1977; Niklasson et al., 1995). Occurrence of hantavirus infection in this species was also found to correlate with rodent density (Niklasson et al., 1995). Ecological factors such as climatic conditions, food supply, predators or diseases influence population fluctuations (Krebs and Myers, 1974; Flowerdew and Gardner, 1978) and may directly relate to the occurrence of human disease. The May 1993 outbreak of HPS in the southwestern USA was associated with a change in environmental conditions leading to a profusion of pin˜ on nuts and

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

marked increases in rodent populations (Stone, 1993). The antibody prevalence reached 30% in deer mice trapped in the epidemic region in spring 1993 (Childs et al., 1994). In Belgium and in France, mild climatic conditions and an unusual abundance of beechnuts followed by a high density of red bank voles were also observed during the 1992 – 1993 and the 1995 – 1996 outbreaks of HFRS (Clement et al., 1994a; Le Guenno et al., 1994). Most cases were registered in a region on both sides of the Franco-Belgian border, where mixed pine and broad leaf forests dominate and constitute the preferred environment for the red bank voles.

Although a high prevalence of hantavirus infection in rodents and an unusually high population density are generally reported during epidemic years, there is limited knowledge of the dynamics of infection in small mammal communities. Recent field studies showed that infection rates in deer mice might be independent of population density and that the virus activity might be focal and unevenly distributed in the host species (Weigler et al., 1996; Graham and Chomel, 1997; Jay et al., 1997; Mills et al., 1997; Boone et al., 1998; Kuenzi et al., 1999). Focality of infection was also observed in red bank voles (Traavik et al., 1984; Verhagen et al., 1986; Ahlm et al., 1997). The landscape features, the seasonal food availability, the rodent community structure and the behaviours of infected mice have been proposed as factors influencing the patchy distribution of hantavirus infection in the reservoir species (Boone et al., 1998; Glass et al., 1998; Abbott et al., 1999; Root et al., 1999). The persistent infection in a few long-lived residents and a threshold in population density have also been suggested recently for the maintenance of the enzootic cycle in the foci (Boone et al., 1998; Abbott et al., 1999; Mills et al., 1999).

Here we report results of a survey undertaken in the Belgian HFRS epidemic region to investigate spatial and temporal variations in prevalence of hantavirus infection among rodents. Dynamics of populations were determined in four different sites by use of a live-trapping method.

Materials and methods

SURVEY SITES AND RODENT COLLECTION

To estimate the prevalence of hantavirus infection in conjunction with rodent population densities, four sites located in Thuin, Montbliart, Momignies and Couvin were selected for a mark-recapture survey. During three consecutive years, rodent communities were sampled in fall, when the population reaches a peak and is mainly represented by juveniles and subadults, and in spring, characterised by a declined overwintered adult population. To provide replicated data for rodent populations, we selected an array of habitats suitable for the red bank voles. The trapping sites were located in broad leaf or mixed pine and broad leaf areas, with a dense ground vegetation essentially constituted by brambles (*Rubus* sp.). In each site, a 0.81-ha area was marked by stakes placed at 10-m intervals. Rodent density was estimated by the enumeration method (Krebs, 1966; Hilborn et al., 1976). The minimum number alive (MNA) at a site represented the total number of marked and released rodents during a trapping session. In each area, 100 livetraps (Sherman Live Trap, Tallahassee, FL and Tomahawk Live Trap, Tomahawk, WI) were placed at 10-m intervals in a 10 × 10 trapping grid for 4 consecutive nights. In Couvin, trappings of fall 1996 were performed on a

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

0.36-ha area with 36 traps spaced 6 m apart on six trap-lines. The traps were baited with peanut butter and cotton wool was provided for protection of the captured animals against the cold. The traps were inspected twice a day, in early morning and late afternoon. Rodents were anaesthetised with isoflurane (Fore`ne, Abbott), individually marked by toe-clipping and a sample of blood was collected from the retro-orbital sinus. Animals were examined for sex and weight and were released at their original place of capture. When a rodent was found dead in a trap or was killed during bleeding, organs (lungs, liver, kidneys, spleen) were placed into cryovials and immediately stored in liquid nitrogen in the field.

In spring 1999, 120 additional traps were used to increase the surface of the mark-recapture sites. These traps were spaced 10 m apart on 12 lines radiating from the edges of the grid, at 30° angles from one another.

From October 1996 to May 1999, trapping of small mammals and collection of samples were conducted in 21 wooded sites distributed in five main localities of the southern epidemic region of Belgium. In each locality, distances between trapping sites did not exceed 2 km. In the 17 trapping sites where mark-recapture was not performed, 23 – 30 livetraps were placed on traplines, at 5-m intervals for 2 – 4 consecutive nights. The animals were sacrificed and blood and organs were collected for serology, PCR analysis and further viral genome sequencing.

SEROLOGICAL SCREENING AND ANTIGEN DETECTION

Sera of rodents trapped between October 1996 and October 1998 were screened by an IgG ELISA using PUU CG18-20, HTN 76-118 and DOB infected Vero E6 cell lysates as viral antigens and non-infected Vero E6 cells as negative control antigen. The 96-well plates were coated with antigen diluted 1:1000 in phosphate buffered saline (PBS) at 4°C overnight. Sera diluted 1:100 in ELISA buffer (PBS with 0.1% Tween 20 and 5% fetal calf serum) were incubated at 37°C for 1 h followed by peroxidase-labelled goat anti-mouse IgG (Sigma) diluted 1:5000 in ELISA buffer. After 1-h incubation at 37°C, TMB substrate (Sigma) was added and the reaction was stopped after 10 min with 1 N H_2SO_4. Plates were washed three times between each step with washing buffer (PBS with 0.1% Tween 20). For all sera, the optical density (OD) was determined on both viral and negative control antigen at 450 nm. The cut-off value was estimated by a log-likelihood ratio method (Parker et al., 1990; Vizard et al., 1990). The natural logarithm of *P/N* (absorbance of viral antigen/absorbance of control antigen) was calculated to obtain a normal distribution of data. The proportion of results from the infected and the non-infected populations was assessed as the mean and variance for each population and the cut-off value which minimised the total number of misdiagnoses (log(*P/N*)= 0.16) was retained. Borderline sera with a log(*P/N*) ranging between 0.15 and 0.21 were also tested by a focus reduction neutralisation test (FRNT) as described by Lundkvist et al. (1997b).

Sera collected in spring 1999 were screened by using a PUU virus IgG ELISA kit (Progen Biotechnik, Heidelberg, Germany). Assays were performed as described in the protocol except that the goat anti-mouse IgG peroxidase conjugate was used in place of the anti-human IgG peroxidase

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

conjugate provided in the kit. The cut-off value was also estimated by a log-likelihood ratio method.

To compare the results obtained with the ELISAs used for serological screening, the sera of 147 C. *glareolus* and 24 *Apodemus sylvaticus* collected in fall 1998 were also tested with the Progen ELISA kit. Both ELISA tests provided the same results with the exception of two C. *glareolus* that were found borderline positive with the first ELISA and seropositive with the Progen kit.

Hantavirus antigen was detected in lung tissues using an antigen-capture ELISA (Hantagnost) as described by Tkachenko et al. (1981).

REVERSE TRANSCRIPTION AND NESTED-PCR AMPLIFICATION

Approximately 30 mg of lung tissue was mechanically homogenised in 1 ml of RNA NOW reagent (Biogentex), a guanidine isothiocyanate solution containing phenol, and total RNA was extracted according to the manufacturer's instructions. RNA (1 – 10 µg) was transcribed to cDNA using 4 U of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim), 50 mM Tris– HCl (pH 8.5), 30 mM KCl, 8 mM $MgCl_2$,0.2 mM (each) dNTPs (Boehringer Mannheim), 0.06 mM random hexamers (GIBCO BRL) and 10 U of placental ribonuclease inhibitor (Pharmacia Biotech). The mixture was incubated at 42°C for 1 h. A nested-PCR strategy was used to amplify the cDNA. A first screening was performed for the amplification of a fragment of 204 bp from the S segment, using genus-specific primers. PUU-specific primers were also used to amplify a fragment of 312 bp from the M segment. The oligonucleotides, designed on the basis of primers sequences described by Pilaski et al. (1994), were (the polarity of the sequence and the nucleotide position of the 5' end of the primer are given in parentheses): S1, TCGATGCCCAC-CAACATG (+ 993); S2, CTTAGCTCGGG-ATCCATGTC (− 1274); S3, GAATTAGGT-GCATTCTTTTCTATATTGC (+ 1033); and S4, GATCTACCATTTCTTTACCCCAT-TC (− 1237) for the S segment and M1, ACCT-GATTGTCCAGGGGTAGG (+ 2375); M2, GGGTTCTGTAACATTGAA-TGATTG (− 2870); M3, CTTTGAGGTACACAAGGAAAG (+ 2467); and M4, GGCGTTGTTGCAAATG-CACAT (− 2779) for the M segment.

A total of 5 µl of cDNA was added to 45 µl of a mixture containing 10 mM Tris– HCl (pH 8.3), 1.5 mM MgCl2, 50 mM KCl, 0.2 mM (each) dNTPs, 1 µM (each) forward and reverse outer primers and 2 U of *Taq* polymerase (Boehringer Mannheim). For the second amplification, 5 µl of the first PCR product was added to 45 µl of the same reaction mixture as above except that inner primers were added instead of outer primers. The nested-PCR for the S fragment was performed in 30 cycles at 95°C for 1 min, 52°C for 45 s and 72°C for 2 min in the first amplification and in 26 cycles at 95°C for 1 min, 55°C for 45 s and 72°C for 2 min in the second amplification. Cycling conditions of the nested-PCR for the M fragment were as follow: 95°C for 1 min, 46°C for 1 min and 72°C for 2 min in 30 and 26 cycles during the first and second amplifications, respectively. The samples were incubated at 72°C for 10 min after each amplification. Mouse β-actin primers (Clontech) were used as an internal control to monitor the efficiency of the extraction procedure, the reverse transcription and the nested-PCR. In first and second PCR amplifications, 0.1 µM of those primers was added to the mixture. A β-actin amplimer of 504 bp was expected for each rodent sample tested. RNA extracted

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

from non-infected Vero E6 cell lysates was used as a negative control for the RT-nested-PCR reactions. PCR products were analysed by electrophoresis in 1.5% agarose gel.

The specificity of both S and M segments oligonucleotides was tested with Vero E6 cells infected with the Belgian strains PUU CG13891, PUU CG18-20, HTN 76-118 and SEO SR-11. An amplimer was obtained for all strains when S primers were used whereas amplification was restricted to PUU CG13891 and PUU CG18-20 with M primers.

Sensitivity of the S and M nested-PCRs was assessed using tenfold dilutions (10 µg to 1 ng) of total RNA preparations. A band was visualised with 10, 1 and 0.1 µg of RNA, with and without addition of mouse β-actin primers.

DIRECT SEQUENCING OF PCR AMPLICONS

Amplified DNA was purified with the Microspin Columns kit (Amersham Pharmacia Biotech) and automatically sequenced by the dideoxy method using a FS polymerase kit (Perkin Elmer) according to the manufacturer's instructions. Sequencing was performed in both directions with primers S3 and S4 for the S segment and M3 and M4 for the M segment.

Field and laboratory safety measures to prevent exposure to PUU virus were followed according to the CDC recommendations (CDC, 1993, 1994).

Results

SEROLOGICAL DATA

From October 1996 to May 1999, a total of 1062 red bank voles, 832 wood mice (A. *sylvaticus*), 31 yellow-necked field mice, 18 shrews (*Sorex* sp.), two field voles (*Microtus agrestis*) and one rat (*Rattus* sp.) were trapped in southern Belgium. Among those, 1000 red bank voles, 804 wood mice and all the yellow-necked field mice and field voles were serologically tested by ELISA. IgG antibodies to PUU were detected in 257 red bank voles. A total of eight wood mice trapped in spring 1999 also reacted to PUU by ELISA. However, further FRNT screening with PUU and other hantaviruses still needs to be performed to determine accurately the strain that infected these animals. No specific DOB-or HTN-seropositivity was recorded. Although 12 red bank voles, 31 wood mice and four yellow-necked field mice trapped between October 1996 and October 1998 showed borderline positive results for DOB and/or HNT by ELISA, all were negative by FRNT. A high seroprevalence (20.1%, 37 of 184) in red bank voles was recorded during the fall of the 1996 HFRS epidemic year. It decreased slightly (14.3%, six of 42) in the declined overwintered population of spring 1997. Despite a subsequent increase in population density, the prevalence in October 1997 (6.6%, 11 of 166) was significantly lower than in the previous fall ($\chi^2 = 13.4$, $P < 0.01$). Persisting low seroprevalences were recorded in spring (6.4%, three of 47) and fall (6.7%, 11 of 165) 1998. The highest seroprevalence (47.7%, 189/396) was recorded during the spring of 1999 and was associated with a marked increase in the population density. The eight seropositive wood mice originated from the grids of Montbliart ($n = 4$), Momignies ($n = 2$) and Couvin ($n = 2$).

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

There were four males and four females and all were adults, with an average weight of 25.4 g.

VIRAL ANTIGEN AND RNA DETECTION

Initially, an antigen-capture ELISA was used for hantavirus detection in rodent lungs. Of 59 red bank voles trapped in fall 1996, four (6.8%) had detectable levels of viral antigen, two being also seropositive. Altogether, 74 red bank voles (trapped in fall 1996 and in spring 1997) were tested both by antigen ELISA and by nested-PCR (Table 1). The detection of viral RNA in seven antigen-negative animals revealed a higher sensitivity of the nested-PCR that was therefore preferably used for tissue screening during the four following trapping seasons. The screening of tissue samples collected in spring 1999 is still under process. A total of 260 small mammals (180 red bank voles, 60 wood mice, 18 shrews, one field vole and one rat) trapped between October 1996 and October 1998 were first tested using the S fragment-specific PCR. Positive samples were then investigated by RT-PCR for the presence of PUU M segment sequences. All positive animals were red bank voles and exhibited both S and M fragments amplification. High infection rates were recorded in fall 1996 and fall 1998 with 16.2% (12 of 74) and 21.2% (seven of 33) of rodents positive, respectively. During the other capture sessions, viral RNA was only detected in two out of 57 (3.5%) red bank voles trapped in fall 1997.

Concordance was high between antibody ELISA and PCR results in red bank voles (Table 1). A total of 122 seronegative animals scored negative by PCR and viral RNA was detected in 11 of 13 seropositive voles. The two seropositive and PCR negative bank voles were trapped in fall 1997. One was a young male weighing 12 g and the other was an adult male of 21 g. Discordant data were also observed in two seronegative animals trapped in fall 1998 that scored positive by PCR. Both were males of 17 g and 20 g, respectively.

Table 1. *Comparison of antibody and antigen ELISAs and nested-PCR results in red bank voles (1996–1998)*[a]

Number tested	Antibody ELISA	Antigen ELISA	Nested-PCR
39	N	N	N
83	N	NT	N
24	NT[b]	N	N
2	P	P	P
2	NT	P	P
3	P	N	P
4	NT	N	P
6	P	NT	P
2	P	NT	N
2	N	NT	P

[a] *N, negative; P, positive; NT, non-tested.* / [b] *Serum not available.*

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

GENETIC DIVERSITY

The sequences of PCR products obtained from samples of four different localities showed a nucleotide divergence of 1.3 – 3.4% for the S segment of PUU and 1.4 – 3.3% for the M segment. The highest sequence differences were observed between the most distant localities. A more detailed genetic analysis of wild-type PUU strains will be published elsewhere.

GEOGRAPHICAL DISTRIBUTION OF POSITIVE RODENTS

Seropositive and/or PCR positive red bank voles were detected at least once in ten of the 21 trapping sites visited over the 3-year survey. Areas where hantavirus infection was present were distributed in four distinct localities (Table 2). In nine of the 11 sites where positive rodents were never detected, less than seven red bank voles were captured, limiting the possibility of finding infected rodents, if present. Of the nine sites sampled in fall 1996, eight were positive for infection. A focal distribution of hantavirus infection was found within the four localities during the seasons when the global prevalence rates were the lowest.

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Table 2. *Results of red bank vole trapping in 21 sites of southern Belgium from October 1996 to May 1999, including the distribution and percentage of sero- and/or PCR-positive animals for hantavirus infection*

Site	Fall 1996			Spring 1997			Fall 1997			Spring 1998			Fall 1998			Spring 1999		
	Trap nightsa	T.I. (%)b	% Pos (n)c	Trap nights	T.I. (%)	% Pos (*n*)	Trap nights	T.I. (%)	% Pos (*n*)	Trap nights	T.I. (%)	% Pos (*n*)	Trap nights	T.I. (%)	% Pos (*n*)	Trap nights	T.I. (%)	% Pos (*n*)
Thuin																		
Site 1																		
Grid	400	24.2	22.5 (71)	400	8.5	23.1 (13)	400	15.2	6.1 (33)	400	9.3	10.0 (10)	400	13.5	0.0 (35)	400	22.7	10.7 (56)
Trap-lines																480	11.2	29.4 (34)
Site 2				48	2.1	0.0 (1)												
Site 3				48	25.3	0.0 (12)	69	21.5	0.0 (18)									
Site 4										92	3.3	0.0 (2)						
Montbliart																		
Site 1																		
Grid	325	14.9	15.2 (33)	400	2.6	28.6 (7)	400	19.2	14.7 (34)	400	6.5	40.0 (5)	400	19.9	0.0 (51)	400	19.3	49.0 (51)
Trap-lines																480	16.1	55.9 (59)
Site 2				48	0.0	0.0 (0)												
Site 3							46	6.6	66.7 (3)				92	15.4	42.9 (14)			
Site 4							46	2.2	0.0 (1)									
Site 5										69	4.3	0.0 (2)						
Momignies																		
Site 1																		
Grid	400	12.9	23.5 (34)	400	0.0	0.0 (0)	400	12.1	0.0 (32)	400	8.2	0.0 (14)	400	17.6	15.7 (51)	400	25.5	63.0 (54)

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Trap-lines																	
Site 2	60	35.1	6.3 (16)	96	6.3	0.0 (6)	92	17.4	7.1 (14)				69	5.9	25.0 (4)		
Site 3	60	24.6	21.4 (14)														
Site 4										69	4.4	0.0 (1)					
Couvin																	
Site 1																	
Grid	72	27.1	33.3 (18)	400	0.3	0.0 (1)	400	9.7	0.0 (26)	400	5.6	0.0 (9)	400	5.4	0.0 (18)	400	10.2	59.3 (27)
Trap-lines																480	17.9	66.7 (60)
Site 2	75	9.7	14.3 (7)															
Site 3	72	18.3	0.0 (12)															
Site 4	72	14.3	12.5 (8)	24	4.2	0.0 (1)	44	2.3	100 (1)									
Site 5				24	4.2	100 (1)	48	15.1	0.0 (6)									
Site 6				24	0.0	0.0 (0)												
Site 7													69	10.3	0.0 (6)			
Dinant										92	9.8	0.0 (4)						
Total	1536	18.3	19.2 (213)	1912	3.0	14.3 (42)	1945	14.0	6.5 (168)	1922	7.1	6.4 (47)	1830	13.7	8.4 (179)	3520	17.3	47.7 (396)

[a] Total number of traps×total number of nights. / [b] Trapping index (%), total number of rodents trapped/(trap nights−1/2 of traps sprung not containing a rodent)×100. / [c] % Positive (number teste

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

COMMUNITY STRUCTURE AND PREVALENCE

As both serum and tissues were not available for all trapped rodents, serological and PCR results were pooled for further epidemiological analysis. For spring 1999, analysis was based on serological data only. The results from all trapping sites were combined.

HANTAVIRUS INFECTION AND RODENT BODY MASS

When bank voles were grouped according to their weight in 3-g intervals, an increased frequency of infection in heavier mass classes was observed. As the weight distribution of infection was similar in males and females, results were pooled for analysis. In fall 1996 and spring 1997, a distinct higher prevalence was recorded in animals over 18 g. The difference between infection rates was highly significant ($P < 0.001$) in fall 1996 when 72.7% (16 of 22) of rodents > 18 g were positive, compared with 13.1% (25 of 191) of animals ≤ 18 g. During the following spring, none of the 13 juveniles and subadults was positive while a prevalence rate of 20.7% (six of 29) was still recorded in animals > 18 g. In fall 1997 and spring and fall 1998, the markedly higher prevalence was observed in rodents > 15 g. In October 1997, though the difference between the two main weight groups was less marked than in the previous fall, the infection rate in rodents > 15 g (9.7%, ten of 103) was significantly ($x2 = 4.35$; $P = 0.04$) higher than in younger animals (1.5%, one of 65). The lowest prevalence in adults was recorded in spring 1998 when only 6.7% (three of 45) of positive rodents were found in the mass classes > 15 g. No infected juveniles were observed at that time. As observed in October 1997, the prevalence in rodents > 15 g in fall 1998 (11.4%, 14 of 123) was significantly ($\chi2 = 4.62$; $P = 0.03$) higher than in animals ≤ 15 g (1.8%, one of 56). Contrary to the previous springs, seropositive animals in May 1999 were observed in all mass classes. The prevalence in animals < 10 g (66.7%, 12 of 18) was significantly higher ($P < 0.001$) than in rodents in the weight range 10 – 18 g (26.8%, 52 of 194). The seroprevalence in adults > 18 g (67.9%, 125 of 184) was also significantly ($P < 0.001$) higher than in animals of lower mass classes (30.2%, 64 of 212).

VARIATION OF PREVALENCE WITH SEX

In fall 1996, males and females were similarly infected with high prevalence rates of 19.4% (20 of 103) and 19.1% (21 of 110), respectively. During the following spring, 12.5% (three of 24) of males and 16.7% (three of 18) of females were still found positive for infection. In fall 1997, a higher prevalence was recorded in males (8.6%, ten of 116; infection rate in females: 1.9%, one of 52) which were also more abundant as evidenced by a sex ratio of 2.2. However, the difference was not statistically significant ($\chi2 = 2.63$, $P = 0.10$). In spring 1998, the infection was more evenly distributed between sexes with prevalence rates of 7.4% (two of 27) in males and 5.0% (one of 20) in females. A higher infection rate in males (12.0%, nine of 75) than in females (5.8%, six of 104) was again observed in fall 1998 but the difference was not statistically significant ($\chi2 = 2.20$, $P = 0.14$). In spring 1999, males and females were highly and similarly infected with prevalence rates of 45.3% (97 of 214) and 50.5% (92 of 182), respectively.

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

POPULATION AND INFECTION DYNAMICS

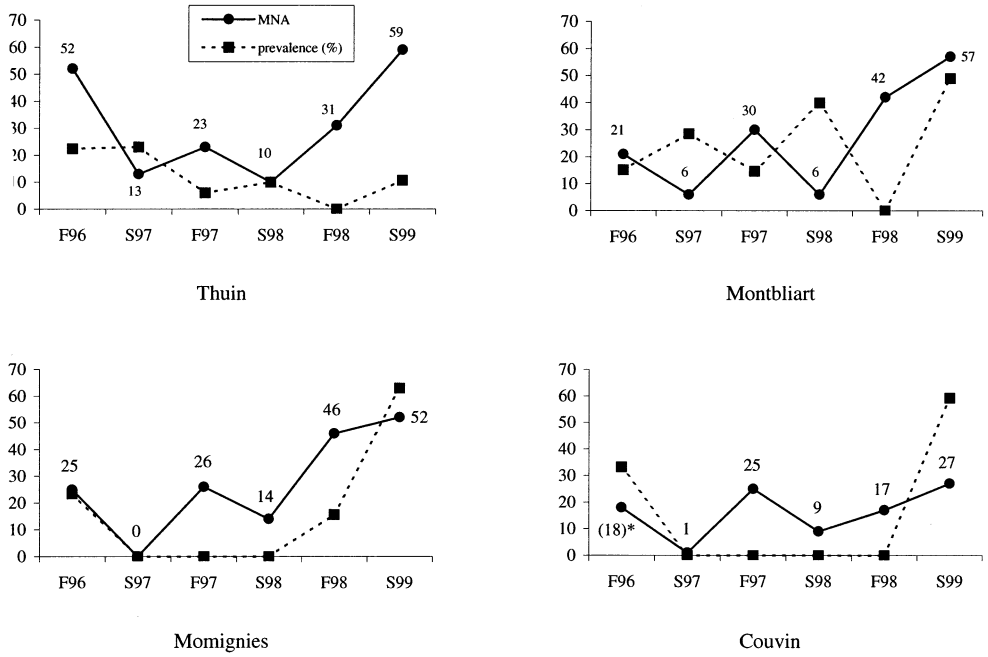
In Fig. 1, the prevalence of hantavirus infection in the four 0.81-ha mark-recapture grids is presented in parallel with the minimum number of rodents alive during each trapping session. In fall 1996, high MNA values and a high prevalence with a minimum of five infected rodents per site was recorded in all four localities. During the following spring, bank vole populations declined drastically in Momignies and Couvin with none and one animal/grid, respectively. A prevalence exceeding 20% was observed in both other sites where the population density did not fall under six voles/grid. In fall 1997, rodent densities increased in all localities. However, no positive animal was found in either site where a severe population decline occurred in the previous spring. In spring 1998, despite the absence of a population crash, a zero-prevalence was again recorded in Momignies and in Couvin whereas infected bank voles were still found in both other sites. In fall 1998, no positive rodent was found in Thuin and Montbliart, despite the high population density. Hantavirus infection was only detected in bank voles trapped in Momignies. No population decline was observed during the following spring. High MNA values ranging between 27 and 59 individuals/0.81 ha were concomitant with high seroprevalences in all grids. The minimum of positive animals (n = 6) per site was recorded in Thuin. A total of 25 and 34 positive rodents were observed in Montbliart and Momignies, respectively.

Prevalence data according to the sex of bank voles in the four grids are presented in Table 3. In fall 1996, both sexes were similarly infected in all sites. In contrast with females, positive males were constantly found in Thuin and Montbliart during the three following trapping seasons. Moreover, the high prevalence recorded in Montbliart through these seasons was concomitant with a systematically higher proportion of males in the population. On the other hand, males were also more abundant in both negative sites in fall 1997 and in spring 1998. Males and females were similarly infected in the only positive site in fall 1998. Couvin excepted, females were also more abundant during this season. Similar high prevalence rates were recorded in both sexes during the following spring when males and females were globally equally represented.

In fall 1996, infection rates ; ≥ 50% were found in animals > 18 g in all sites whereas the frequency of positive juveniles and subadults did not exceed 26.7% (four of 15) (Table 4). In spring 1997, the highest prevalence in adults (42.9%, three of 7) was recorded in the site of Thuin, where juveniles and subadults were the most abundant and exhibited a high prevalence (18.8%, 12 of 64) during the previous fall. However, data from the following trapping seasons did not seem to indicate the existence of a relation between the density and prevalence in juveniles in fall and the occurrence of infection in the overwintered population. Thuin excepted, the infection rates in animals > 18 g in spring 1999 were similar to those observed in the same mass class during the fall of the 1996 HFRS epidemic year.

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Figure 1. *Red bank voles densities and prevalence of hantavirus infection in the four mark-recapture sites through 1996 – 1999*



*Prevalence (%), (number positive/number tested) × 100. F, fall; MNA, number of marked and released rodents during a 4-night session; S, spring. * In fall 1996, live-trapping in Couvin was performed on a 0.36-ha area*

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Table 3. *Sex distribution of hantavirus infection in bank voles from the four grids*[a]

Grid	Fall 1996			Spring 1997			Fall 1997			Spring 1998			Fall 1998			Spring 1999		
	M	F	S.R.	M	F	S.R.	M	F	S.R.	M	F	S.R.	M	F	S.R.	M	F	S.R.
Thuin	28.1 (32)[b]	17.9 (39)	0.8	20.0 (5)	25.0 (8)	0.6	11.1 (18)	0.0 (15)	1.2	14.3 (7)	0.0 (3)	2.3	0.0 (17)	0.0 (18)	0.9	8.7 (23)	12.1 (33)	0.7
Montbliart	16.7 (24)	11.1 (9)	2.7	28.6 (7)	0.0 (0)	–	16.7 (24)	10.0 (10)	2.4	33.3 (3)	50.0 (2)	1.5	0.0 (18)	0.0 (33)	0.5	42.9 (28)	56.5 (23)	1.2
Momignies	28.6 (14)	20.0 (20)	0.7	0.0 (0)	0.0 (0)	–	0.0 (27)	0.0 (5)	5.4	0.0 (9)	0.0 (5)	1.8	15.0 (20)	16.1 (31)	0.6	63.3 (30)	62.5 (24)	1.3
Couvin	20.0 (5)	38.5 (13)	0.4	0.0 (0)	0.0 (1)	–	0.0 (20)	0.0 (6)	3.3	0.0 (5)	0.0 (4)	1.3	0.0 (9)	0.0 (9)	1.0	55.6 (18)	66.7 (9)	2.0
Total	24.0 (75)	20.1 (81)	0.9	25.0 (12)	22.2 (9)	1.3	6.7 (89)	2.8 (36)	2.5	8.3 (24)	7.1 (14)	1.7	4.7 (64)	5.5 (91)	0.7	43.4 (99)	42.7 (89)	1.1

[a] *F, females; M, male; S.R., sex ratio. /* [b] *% Positive (number tested).*

Table 4. *Influence of weight on prevalence in rodents from the live-trapping sites*

Grid	Fall 1996		Spring 1997		Fall 1997		Spring 1998		Fall 1998		Spring 1999	
	≤18 g	**>18 g**	**≤18 g**	**>18 g**	**≤15 g**	**>15 g**	**≤15 g**	**>15 g**	**≤15 g**	**>15 g**	**≤18 g**	**>18 g**
Thuin	18.8 (64)[a]	57.1 (7)	0.0 (6)	42.9 (7)	0.0 (16)	11.8 (17)	0.0 (0)	10.0 (10)	0.0 (12)	0.0 (23)	6.7 (30)	15.4 (26)
Montbliart	10.3 (29)	50.0 (4)	0.0 (0)	28.6 (7)	0.0 (7)	18.5 (27)	0.0 (0)	40.0 (5)	0.0 (18)	0.0 (33)	37.5 (40)	90.9 (11)
Momignies	7.1 (28)	100 (6)	0.0 (0)	0.0 (0)	0.0 (10)	0.0 (22)	0.0 (1)	0.0 (13)	5.9 (17)	20.6 (34)	50.0 (30)	79.2 (24)
Couvin	26.7 (15)	66.7 (3)	0.0 (0)	0.0 (1)	0.0 (18)	0.0 (8)	0.0 (1)	0.0 (8)	0.0 (3)	0.0 (15)	55.6 (18)	66.7 (9)
Total	15.4 (136)	70.0 (20)	0.0 (6)	33.3 (15)	0.0 (51)	9.5 (74)	0.0 (2)	8.3 (36)	2.0 (50)	6.7 (105)	35.6 (118)	55.7 (70)

[a] *% Positive (number tested).*

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Discussion

HFRS cases reported in Belgium have been attributed so far to PUU virus (Clement et al., 1994a; Heyman et al., 1999). Although the red bank vole has been identified as the main reservoir for hantavirus in Belgium, four other rodent species (M. *agrestis, Ondatra zibethicus*, *A. sylvaticus* and *R. norvegicus*) and two insectivores (*Sorex araneus* and *Talpa europea*) have also been sporadically reported positive for the presence of hantavirus antigen and/or antibodies (Verhagen et al., 1987; Clement et al., 1994b).

In the present study, evidence of PUU infection was restricted to the red bank voles. The detection of eight seropositive wood mice for hantavirus infection and the borderline ELISA results for HTN and/or DOB obtained mainly in *Apodemus* species might suggest the presence of a still unknown hantavirus strain in this species.

Temporal and spatial variations in prevalence of infection were observed among bank voles within the investigated area. High prevalence rates were recorded in fall 1996 and spring 1999. An apparent widespread distribution of the virus also characterised the fall of the 1996 HFRS epidemic year whereas lower infection rates and a focal occurrence of positive rodents were observed during the four next trapping periods.

The unusually favourable ecological conditions (profusion of beechnuts and mild weather) observed in 1995 – 1996 might have enabled populations to achieve higher fall densities and over-winter survival. The expanded population might have contributed to a more effective dissemination of the virus, also allowing infected animals to disperse in new microhabitats. Although no trapping was performed in spring 1996, the high proportion of positive sites, as well as the high seroprevalence in adults and the abundance of juveniles and subadults recorded in fall 1996 provide supportive evidence for that hypothesis. Moreover, the concordance between serological and PCR results suggests that seropositive animals were also infectious during that period. The subsequent decline in the rodent population and the low survival of older animals over the winter might have limited the spread of the virus during the following year, resulting in lower prevalence rates and in a focal distribution of infected rodents. In the absence of a marked increase in population density, the transmission rate of the virus remained low until the fall of 1998. As recorded in 1995 – 1996, favourable environmental conditions (abundance of acorns and mild weather) were again observed in fall 1998 and during the following winter. These conditions resulted in a high over-winter survival of the rodents and in a rapid onset of the breeding season in early spring or possibly an extension of the reproductive activities over the winter. The increased population density involved higher rates of transmission of the virus, as revealed in May 1999 by the very high seroprevalence (47.7%) in red bank voles. The possible occurrence of spillover to wood mice during this season might also reflect the increased spread of PUU virus. The detection of viral RNA in two seronegative bank voles and the high proportion of PCR positive rodents (21.2%) in fall 1998 even suggest that the extended spread of the virus started during this season. Despite the high seroprevalence in bank voles and their unusual abundance in spring 1999, no

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

HFRS outbreak was reported during the following months. However, more epidemiological data are still needed to exclude with certainty the risk of a new epidemic in Belgium.

Data collected from the four mark-recapture grids showed that rodent density and infection rates were high in all sites during the 1996 HFRS epidemic period (Fig. 1). In two places, the severity of the 1997 spring decline resulted in an apparent extinction of the virus, despite a subsequent increase of the host population size. In contrast, positive animals were constantly detected until fall 1998 in both other grids where the recorded MNA values were never under six animals/grid. Our data are in agreement with a study on SN virus where sites with seven or more deer mice were significantly more likely than low-density sites to have seropositive animals present (Boone et al., 1998). Taken together, these observations suggest that a threshold in population size is crucial for the maintenance of the enzootic cycle.

Although a minimum density of bank voles may be required for an effective spread of the virus, data from fall 1998 showed that infected animals may be absent in high-density sites. The dynamics of infection may therefore be influenced by other factors such as particular habitat types, ecological conditions or community structure parameters (Xu et al., 1985; Verhagen et al., 1987; Mills et al., 1997; Abbott et al., 1999; Mills et al., 1999; Root et al., 1999). Except for fall 1996 and spring 1999 when the higher prevalence rates were related to the irruption in rodent densities, there was no apparent relation between the prevalence and the relative abundance of rodents. On the contrary, data from two grids showed that the prevalence in the declined overwintered population was constantly higher than in the previous crowded fall population. This observation may be explained by the changing age structure towards juveniles in fall that would decrease the global prevalence rate. A similar pattern was observed in USA where the prevalence of hantavirus infection over time was inversely related to the population density of brush mice (*Peromyscus boylii*) and deer mice (Boone et al., 1998; Abbott et al., 1999; Calisher et al., 1999). In northern Sweden, populations of voles fluctuate on a 3 – 4-year cycle and animals may be 300 times more abundant during peak periods (Hansson and Henttonen, 1985; Ho¨ rnfeldt, 1994). In fall, a correlation between infection rates in red bank voles and the relative rodent density was reported whereas in spring, the prevalence was correlated with the abundance of rodents in the previous fall and spring (Niklasson et al., 1995). Although populations of red bank voles are non-cyclic in western Europe, data from our mark-recapture grids seem to indicate a delayed density-dependence (DDD) of prevalence in spring. Datasets on prevalence of hantavirus infection in *Peromyscus* sp. in southwestern USA also agree with the DDD model that provides enlightenment on the interaction of the dynamics of infection and population densities (Mills et al., 1999).

Sex and age are both physiological factors implicated in the dynamics of infection. An increased frequency of infected animals in heavier mass classes is generally reported in hantavirus reservoir hosts, supporting the model wherein the virus is transmitted horizontally. This suggests that intraspecies behaviour like communal nesting, grooming or wounding by bites and scratching during aggressive encounters are important in the acquisition of infection that generally occurs at the onset of sexual maturity (Verhagen et al., 1986; Childs et al., 1987a,b; Niklasson et al., 1995). Infection with SN or SEO viruses was also clearly associated more frequently with heavier males,

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

supposedly because of fighting and of larger home range, leading to an increased risk of exposure to virus (Childs et al., 1985; Glass et al., 1988; Childs et al., 1994; Otteson et al., 1996; Mills et al., 1997, 1998; Kuenzi et al., 1999). In our study, the systematic detection of infected adult males in both grids found positive until spring 1998 corroborates these observations (Table 3). Our results are also in agreement with a recent study in USA where foci of hantavirus infection were apparently sustained through the presence of persistently infected long-lived *Peromyscus* males (Abbott et al., 1999). Although the differences were not statistically significant, global prevalence rates were higher in males than in females in fall, except during the 1996 epidemic year. In contrast, both sexes were similarly infected in fall 1996 and in spring. These data suggest that irruption in rodent densities and communal nesting during the winter tend to equalize the rates of transmission of the virus between males and females. During each trapping period, adult rodents were more often infected than younger animals. The weight limit for distinct infection rates varied over time. This fluctuation might be explained by the annual variation in the weight of voles or by a delay in the onset of their sexual maturity during the years of peak rodent density (Krebs and Myers, 1974), as observed in 1996 and 1999. Except in fall 1996 and spring 1999, young animals were infrequently infected. The high seroprevalence in rodents < 10 g in spring 1999 probably reflects the transmission of maternal antibodies to offspring, resulting from the high proportion of infected adults during this season. The decreasing prevalence from smallest to largest subclass in juveniles represents the loss of passive immunity until infection occurs at a later age. A similar pattern of infection was observed with SN and Black Creek Canal viruses in deer mice and cotton rats (*Sigmodon hispidus*), respectively (Mills et al., 1997; Glass et al., 1998).

Nucleotide differences between localities within a maximum radius of 40 km were 1.3 – 3.4% for the S segment and 1.9 – 3.4% for the M segment. This observation seems to be in agreement with previously published data on wild PUU viruses in Finland, Russia and Sweden (Plyusnin et al., 1995; Horling et al., 1996) but requires further investigation, especially in more variable regions of the genome.

In conclusion, repeated trapping of rodents in different localities of the Belgian HFRS epidemic region provided evidence that the prevalence of hantavirus infection in bank voles varies considerably over time and space. Except during years of exceptionally high population densities when the virus was widely distributed within the studied area, infected animals occurred in foci such that the infection was always present regionally. The mark-recapture studies conducted twice a year in four grids enabled us to show that a threshold in rodent population size might be required for the maintenance of the enzootic cycle. A long-term study in the same trapping sites should address the effects of ecological and community structure factors on the dynamics of hantavirus infection in rodent hosts.

ACKNOWLEDGEMENTS :

We are indebted to Professor G. van der Groen and G. Beelaert for providing hantavirus strains. We thank Drs C. Lecomte, P. Colson and P. Dehan for useful advice. We also thank J. Tahon, G.

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Latteur and Cdt C. Vandenvelde for helpful cooperation. The computer program for the cut-off value determination in serological analysis was kindly provided by P. Jetteur. This work was financially supported by grants from the Fonds National Belge de la Recherche Scientifique (F.N.R.S), the Belgian Ministry of Defence (Project JSM-R&T, G96/01), the EC (Contract No. BMH4-CT97-2499) and the Swedish Medical Research Council (Projects No. 12177 and 12642).

References

Abbott, K.D., Ksiazek, T.G., Mills, J.N., 1999. Long-term hantavirus persistence in rodent populations in Central Arizona. Emerg. Infect. Dis. 5, 102 – 112.

Ahlm, C., Alexeyev, O.A., Elgh, F., Aava, B., Wadell, G., Tarnvik, A., Juto, P., Palo, T., 1997. High prevalence of hantavirus antibodies in bank voles (*Clethrionomys glareolus*) captured in the vicinity of households afflicted with nephropathia epidemica. Am. J. Trop. Med. Hyg. 56, 674 – 678.

Antic, D., Kang, C.Y., Spik, K., Schmaljohn, C., Vapalahti, O., Vaheri, A., 1992. Comparison of the deduced gene products of the L, M and S genome segments of hantaviruses. Virus Res. 24, 35 – 46.

Avsic-Zupanc, T., Xiao, S.Y., Stojanovic, R., Gligic, A., van der Groen, G., LeDuc, J.W., 1992. Characterization of Dobrava virus: a Hantavirus from Slovenia, Yugoslavia. J. Med. Virol. 38, 132 – 137.

Avsic-Zupanc, T., Petrovec, M., Furlan, P., Kaps, R., Elgh, F., Lundkvist, A0 ., 1999. Hemorrhagic fever with renal syndrome in the Dolenjska region of Slovenia — a 10-year survey. Clin. Infect. Dis. 28, 860 – 865.

Boone, J.D., Otteson, E.W., Mc Gwire, K.C., Villard, P., Rowe, J.E., St Jeor, S.C., 1998. Ecology and demographics of hantavirus infections in rodent populations in the Walker River Basin of Nevada and California. Am. J. Trop. Med. Hyg. 59, 445 – 451.

Brummer-Korvenkontio, M., Vaheri, A., Hovi, T., von Bonsdorff, C.H., Vuorimies, J., Manni, T., Penttinen, K., OkerBlom, N., Lahdevirta, J., 1980. Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. J. Infect. Dis. 141, 131 – 134.

Calisher, C.H., Sweeney, W., Mills, J.N., Beaty, B.J., 1999. Natural history of Sin Nombre virus in western Colorado. Emerg. Infect. Dis. 5, 126 – 134.

CDC, 1993. Hantavirus infection – southwestern United States: interim recommendations for risk reduction. Morbid. Mortal. Weekly Rep. (MMWR) 42, 1 – 13.

CDC, 1994. Laboratory management of agents associated with hantavirus pulmonary syndrome: interim biosafety guidelines. Morbid. Mortal. Weekly Rep. (MMWR) 43, 1 – 7.

Childs, J.E., Korch, G.W., Smith, G.A., Terry, A.D., LeDuc, J.W., 1985. Geographical distribution and age related prevalence of antibody to Hantaan-like virus in rat populations of Baltimore, Maryland, USA. Am. J. Trop. Med. Hyg. 34, 385 – 387.

Childs, J.E., Glass, G.E., Korch, G.W., LeDuc, J.W., 1987a. Prospective seroepidemiology of hantaviruses and population dynamics of small mammal communities of Baltimore, Maryland. Am. J. Trop. Med. Hyg. 37, 648 – 662.

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Childs, J.E., Korch, G.W., Glass, G.E., LeDuc, J.W., Shah, K.V., 1987b. Epizootiology of Hantavirus infections in Baltimore: isolation of a virus from Norway rats, and characteristics of infected rat populations. Am. J. Epidemiol. 126, 55 – 68.

Childs, J.E., Ksiazek, T.G., Spiropoulou, C.F., Krebs, J.W., Morzunov, S., Maupin, G.O., Gage, K.L., Rollin, P.E., Sarisky, J., Enscore, R.E., Frey, J.K., Peters, C.J., Nichol, S.T., 1994. Serologic and genetic identification of Peromyscus maniculatus as the primary rodent reservoir for a new hantavirus in the southwestern United States. J. Infect. Dis. 169, 1271 – 1280.

Clement, J., Mc Kenna, P., Colson, P., Damoiseaux, P., Penalba, C., Halin, P., Lombart, D., 1994a. Hantavirus epidemic in Europe, 1993. Lancet 343, 114.

Clement, J., Mc Kenna, P., Leirs, H., Verhagen, R., Lefevre, A., Song, G., Tkachenko, E.A., van der Groen, G., 1994b. Hantavirus infections in rodents. In: Osterhaus, A.D.M.E. (Ed.), Virus Infections of Rodents and Lagomorphs, vol. 44. Elsevier Science, Amsterdam, pp. 293 – 316.

Duchin, J.S., Koster, F.T., Peters, C.J., Simpson, G.L., Tempest, B., Zaki, S.R., Ksiazek, T.G., Rollin, P.E., Nichol, S., Umland, E.T., Moolenaar, R., Reef, S., Nolte, K., Gallagher, M., Butler, J., Breiman, R., 1994. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. New Engl. J. Med. 330, 949 – 955.

Elliott, R.M., 1990. Molecular biology of the Bunyaviridae. J. Gen. Virol. 71, 501 – 522.

Flowerdew, J.R., Gardner, G., 1978. Small rodent populations and food supply in a Derbyshire ashwood. J. Anim. Ecol. 47, 725 – 740.

Gajdusek, D.C., 1962. Virus hemorrhagic fevers. J. Pediatr. 60, 841 – 857.

Gavrilovskaya, I.N., Apekina, N.S., Bernshtein, A.D., Demina, V.T., Okulova, N.M., Myasnikov, Y.A., Chumakov, M.P., 1990. Pathogenesis of hemorrhagic fever with renal syndrome virus infection and mode of horizontal transmission of hantavirus in bank voles. Arch. Virol., Suppl. 1, S57 – S62.

Glass, G.E., Childs, J.E., Korch, G.W., LeDuc, J.W., 1988. Association of intraspecific wounding with hantaviral infection in wild rats (*Rattus norvegicus*). Epidemiol. Infect. 101, 459 – 472.

Glass, G.E., Livingstone, W., Mills, J.N., Hlady, W.G., Fine, J.B., Biggler, W., Coke, T., Frazier, D., Atherley, S., Rollin, P.E., Ksiazek, T.G., Peters, C.J., Childs, J.E., 1998. Black Creek Canal virus infection in Sigmodon hispidus in southern Florida. Am. J. Trop. Med. Hyg. 59, 699 – 703.

Graham, T.B., Chomel, B.B., 1997. Population dynamics of the deer mouse (*Peromyscus maniculatus*) and Sin Nombre virus, California Channel Islands. Emerg. Infect. Dis. 3, 367 – 370.

Hansson, L., Henttonen, H., 1985. Gradients in density variations of small rodents: the importance of latitude and snow cover. Oecologia 67, 394 – 402.

Heyman, P., Vervoort, T., Colson, P., Chu, Y.-K., Avsic-Zupanc, T., Lundkvist, A., 1999. A major outbreak of hantavirus infection in Belgium in 1995 and 1996. Epidemiol. Infect. 122, 447 – 453.

Hilborn, R., Redfield, J.A., Krebs, C.J., 1976. On the reliability of enumeration for mark and recapture census of voles. Can. J. Zool. 54, 1019 – 1024.

Ho¨ rling, J., Lundkvist, A0 ., Jaarola, M., Plyusnin, A., Tegelstrom, H., Persson, K., Lehvaslaiho, H., Ho¨ rnfeldt, B., Vaheri, A., Niklasson, B., 1996. Distribution and genetic heterogeneity of Puumala virus in Sweden. J. Gen. Virol. 77, 2555 – 2562.

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Ho¨ rnfeldt, B., 1994. Delayed density dependence as a determinant of vole cycles. Ecology 75, 791 – 806.

Jay, M., Ascher, M.S., Chomel, B.B., Madon, M., Sesline, D., Enge, B.A., Hjelle, B., Ksiazek, T.G., Rollin, P.E., Kass, P.H., Reilly, K., 1997. Seroepidemiologic studies of hantavirus infection among wild rodents in California. Emerg. Infect. Dis. 3, 183 – 190.

Krebs, C.J., 1966. Demographic changes in fluctuating populations of Microtus californicus. Ecol. Monogr. 36, 239 – 273.

Krebs, C.J., Myers, J.H., 1974. Population cycles in small mammals. Adv. Ecol. Res. 8, 267 – 399.

Kuenzi, A.J., Morrison, M.L., Swann, D.E., Hardy, P.C., Downard, G.T., 1999. A longitudinal study of Sin Nombre virus prevalence in rodents, Southeastern Arizona. Emerg. Infect. Dis. 5, 113 – 117.

LeDuc, J.W., 1987. Epidemiology of Hantaan and related viruses. Lab. Anim. Sci. 37, 413 – 418.

LeDuc, J.W., Smith, G.A., Bagley, L.R., Hasty, S.E., Johnson, K.M., 1982. Preliminary evidence that Hantaan or a closely related virus is enzootic in domestic rodents. New Engl. J. Med. 307, 624.

Le Guenno, B., Camprasse, M.A., Guilbaut, J.C., Lanoux, P., Hoen, B., 1994. Hantavirus epidemic in Europe, 1993. Lancet 343, 114 – 115.

Lee, H.W., Lee, P.W., Johnson, K.M., 1978. Isolation of the etiologic agent of Korean hemorrhagic fever. J. Infect. Dis. 137, 289 – 308.

Lee, H.W., Baek, L.J., Johnson, K.M., 1982. Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic fever, from wild urban rats. J. Infect. Dis. 146, 638 – 644.

Lundkvist, A0 ., Apekina, N., Myasnikov, Y., Vapalahti, O., Vaheri, A., Plyusnin, A., 1997a. Dobrava hantavirus outbreak in Russia. Lancet 350, 781 – 782.

Lundkvist, A0 ., Hukic, M., Ho¨ rling, J., Gilljam, M., Nichol, S., Niklasson, B., 1997b. 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. 53, 51 – 59.

Lundkvist, A0 ., Vasilenko, V., Golovljova, I., Plyusnin, A., Vaheri, A., 1998. Human Dobrava hantavirus infections in Estonia. Lancet 352, 369.

Lyubsky, S., Gavrilovskaya, I., Luft, B., Mackow, E., 1996. Histopathology of Peromyscus leucopus naturally infected with pathogenic NY-1 hantaviruses: pathologic markers of HPS viral infection in mice. Lab. Invest. 74, 627 – 633.

Mertz, G.J., Hjelle, B.L., Bryan, R.T., 1998. Hantavirus infection. Dis. Mon. 44, 85 – 138.

Mills, J.N., Ksiazek, T.G., Ellis, B.A., Rollin, P.E., Nichol, S.T., Yates, T.L., Gannon, W.L., Levy, C.E., Engelthaler, D.M., Davis, T., Tanda, D.T., Frampton, J.W., Nichols, C.R., Peters, C.J., Childs, J.E., 1997. 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. 56, 273 – 284.

Mills, J.N., Johnson, J.M., Ksiazek, T.G., Ellis, B.A., Rollin, P.E., Yates, T.L., Mann, M.O., Johnson, M.R., Campbell, M.L., Miyashiro, J., Patrick, M., Zyzak, M., Lavender, D., Novak, M.G., Schmidt, K., Peters, C.J., Childs, J.E., 1998. A survey of hantavirus antibody in small-mammal populations in selected United States National Parks. Am. J. Trop. Med. Hyg. 58, 525 – 532.

Mills, J.N., Ksiazek, T.G., Peters, C.J., Childs, J.E., 1999. Long-term studies of hantavirus reservoir populations in the southwestern United States: a synthesis. Emerg. Infect. Dis. 5, 135 – 142.

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Netski, D., Thran, B.H., St Jeor, S.C., 1999. Sin Nombre virus pathogenesis in Peromyscus maniculatus. J. Virol. 73, 585 – 591.

Nichol, S.T., Spiropoulou, C.F., Morzunov, S., Rollin, P.E., Ksiazek, T.G., Feldmann, H., Sanchez, A., Childs, J., Zaki, S., Peters, C.J., 1993. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 262, 914 – 917.

Niklasson, B., LeDuc, J.W., 1987. Epidemiology of nephropathia epidemica in Sweden. J. Infect. Dis. 155, 269 – 276.

Niklasson, B., Ho¨ rnfeldt, B., Lundkvist, A0 ., Bjo¨ rsten, S., Leduc, J., 1995. Temporal dynamics of Puumala virus antibody prevalence in voles and of nephropathia epidemica incidence in humans. Am. J. Trop. Med. Hyg. 53, 134 – 140.

Nystro¨ m, K., 1977. 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. 609, 1 – 92.

Otteson, E.W., Riolo, J., Rowe, J.E., Nichol, S.T., Ksiazek, T.G., Rollin, P.E., St.Jeor, S.C., 1996. Occurrence of hantavirus within the rodent population of northeastern California and Nevada. Am. J. Trop. Med. Hyg. 54, 127 – 133. Parker, R.A., Erdman, D.D., Anderson, L.J., 1990. Use of mixture models in determining laboratory criterion for identification of seropositive individuals: application to parvovirus B19 serology. J. Virol. Methods 27, 135 – 144.

Parmenter, R., Vigil, R., 1993. The Hantavirus Epidemic in the Southwest: An Assessment of Autumn Rodent Densities and Population Demographics in Central and Northern New Mexico. Report to the Federal Centers for Disease Control and Prevention. Federal Centers for Disease Control and Prevention, Atlanta, GA.

Pilaski, J., Feldmann, H., Morzunov, S., Rollin, P.E., Ruo, S.L., Lauer, B., Peters, C.J., Nichol, S.T., 1994. Genetic identification of a new Puumala virus strain causing severe hemorrhagic fever with renal syndrome in Germany. J. Infect. Dis. 170, 1456 – 1462.

Plyusnin, A., Vapalahti, O., Lehvaslaiho, H., Apekina, N., Mikhailova, T., Gavrilovskaya, I., Laakkonen, J., Niemimaa, J., Henttonen, H., Brummer-Korvenkontio, M., Vaheri, A., 1995. Genetic variation of wild Puumala viruses within the serotype, local rodent populations and individual animal. Virus Res. 38, 25 – 41.

Plyusnin, A., Vapalahti, O., Vaheri, A., 1996. Hantaviruses: genome structure, expression and evolution. J. Gen. Virol. 77, 2677 – 2687.

Plyusnin, A., Vapalahti, O., Vasilenko, V., Henttonen, H., Vaheri, A., 1997. Dobrava hantavirus in Estonia: does the virus exist throughout Europe? Lancet 349, 1369 – 1370.

Root, J.J., Calisher, C.H., Beaty, B.J., 1999. Relationships of deer mouse movement, vegetative structure, and prevalence of infection with Sin Nombre virus. J. Wildlife Dis. 35, 311 – 318.

Schmaljohn, C.S., Dalrymple, J.M., 1983. Analysis of Hantaan virus RNA: evidence for a new genus of Bunyaviridae. Virology 131, 482 – 491.

Schmaljohn, C.S., Hjelle, B., 1997. Hantaviruses: a global disease problem. Emerg. Infect. Dis. 3, 95 – 104.

Schmaljohn, C.S., Hasty, S.E., Dalrymple, J.M., LeDuc, J.W., Lee, H.W., von Bonsdorff, C.H., Brummer-Korvenkontio, M., Vaheri, A., Tsai, T.F., Regnery, H.L., Goldgaber, D., Lee, P.W., 1985. Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. Science 227, 1041 – 1044.

Published in : Virus Research (2000), vol. 67, n°1, pp. 91 – 107
DOI: 10.1016/S0168-1702(00)00136-2
Status : Postprint (Author's version)

Stone, R., 1993. The mouse-pin on nut connection. Science 262, 833.

Tkachenko, E.A., Ivanov, A.P., Dzagurova, T.K., Donets, M.A., Rezapkin, G.V., Leshchinskaya, E.V., Zagidulin, U.M., Ivanova, A.A., Stradukhin, O.V., Mustafin, N.M., Savinova, T.I., 1981. Immunosorbent assays for diagnosis of haemorrhagic fever with renal syndrome. Lancet 2, 257 – 258.

Traavik, T., Sommer, A.I., Mehl, R., Berdal, B.P., Stavem, K., Hunderi, O.H., Dalrymple, J.M., 1984. Nephropathia epidemica in Norway: antigen and antibodies in rodent reservoirs and antibodies in selected human populations. J. Hyg. (Lond.) 93, 139 – 146.

Tsai, T.F., 1987. Hemorrhagic fever with renal syndrome: mode of transmission to humans. Lab. Anim. Sci. 37, 428 – 430.

Verhagen, R., Leirs, H., Tkachenko, E., van der Groen, G., 1986. Ecological and epidemiological data on Hantavirus in bank vole populations in Belgium. Arch. Virol. 91, 193 – 205.

Verhagen, R., van der Groen, G., Ivanov, A., Van Rompaey, J., Leirs, H., Verheyen, W., 1987. Occurrence and distribution of Hantavirus in wild living mammals in Belgium. Acta Virol. 31, 43 – 52.

Vizard, A.L., Anderson, G.A., Gasser, R.B., 1990. Determination of the optimum cut-off value of a diagnostic test. Prev. Vet. Med. 10, 137 – 143.

Weigler, B.J., Ksiazek, T.G., Vandenbergh, J.G., Levin, M., Sullivan, W.T., 1996. Serological evidence for zoonotic hantaviruses in North Carolina rodents. J. Wildlife Dis. 32, 354 – 357.

World Health Organization, 1983. Haemorrhagic fever with renal syndrome: memorandum from a WHO meeting. Bull. Who 61, 269 – 275.

Xu, Z.Y., Guo, C.S., Wu, Y.L., Zhang, X.W., Liu, K., 1985. Epidemiological studies of hemorrhagic fever with renal syndrome: analysis of risk factors and mode of transmission. J. Infect. Dis. 152, 137 – 144.

Yanagihara, R., Gajdusek, D.C., 1988. Hemorrhagic fever with renal syndrome: a historical perspective and review of recent advances. In: Gears, J.H.S. (Ed.), CRC Handbook of Viral and Rickettsial Hemorrhagic Fevers. CRC, Boca Raton, FL, pp. 151 – 188.

Yanagihara, R., Amyx, H.L., Gajdusek, D.C., 1985. Experimental infection with Puumala virus, the etiologic agent of nephropathia epidemica in bank voles (*Clethrionomys glareolus*). J. Virol. 55, 34 – 38.

Young, J.C., Mills, J.N., Enria, D.A., Dolan, N.E., Khan, A.S., Ksiazek, T.G., 1998. New world hantaviruses. Br. Med. Bull. 54, 659 – 673.