Thesis for doctoral degree (Ph.D.) 2008

HANTAVIRUSES - SHEDDING, STABILITY AND INDUCTION OF APOPTOSIS

Jonas Hardestam

Karolinska Institutet

Thesis for doctoral degree (Ph.D.) 2008

HANTAVIRUSES - SHEDDING, STABILITY AND INDUCTION OF APOPTOSIS

Jonas Hardestam

Karolinska Institutet

From the Department of Microbiology, Tumor and Cell Biology,
Karolinska Institutet
and the Swedish Institute for Infectious Disease Control,
Stockholm, Sweden

HANTAVIRUSES - SHEDDING, STABILITY AND INDUCTION OF APOPTOSIS

Jonas Hardestam

Karolinska Institutet

Stockholm 2008

Published by Karolinska Institutet.

ISBN 987-91-7409-007-9

Printed by

www.reproprint.se
Gårdsvägen 4, 169 70 Solna

ABSTRACT

Hantaviruses are spread and maintained in different rodent and insectivore species worldwide. Humans are believed to be infected mainly by inhalation of aerosolized contaminated rodent excreta. The natural hosts are generally unaffected by the virus, whereas infection of humans can result in either hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, or hantavirus cardiopulmonary syndrome (HCPS) in the Americas. Infection can also be asymptomatic and the severity of disease partly depends on the hantavirus causing the infection. An increased capillary permeability is the main manifestation for the disease in humans, but the exact mechanisms behind the pathogenesis are unclear. In Sweden and other parts of northern Europe, Puumala (PUUV) hantavirus causes a relatively mild form of HFRS called nephropathia epidemica (NE).

In this thesis, we have followed hantavirus shedding from the host, observed its stability in the environment, studied cytopathogenicity as well as pathogenesis in humans and finally investigated the possibility of transmission between humans.

In detail, we used real-time RT-PCR to study how and when PUUV hantavirus is secreted from the natural host. We observed a clear peak in PUUV-RNA shed in saliva, urine and feces at around 3 weeks after experimental infection of bank voles. Further we showed that all types of excretions were infectious when inoculated intranasally in naïve bank voles, indicating that saliva can transfer the virus via other routes than biting. We also observed a remarkable *ex vivo* stability for Hantaan virus, with infectious virus observed after nearly hundred days incubation at 4ºC. Interestingly, this stability was not exceptional for hantaviruses when compared to vector-borne members of the *Bunyaviridae* family.

Further we wanted to study if the disease in humans could be due to induction of apoptosis and we measured apoptosis both in hantavirus-infected Vero-E6 cells and in hospitalized NE-patients. Increased apoptosis was not observed *in vitro*. However, we found increased levels of serum perforin, granzyme B, and the epithelial cell apoptosis marker caspase cleaved cytokeratin 18 in the patient samples, suggesting that tissue damage is immune-mediated and that apoptosis contributes significantly to the damage.

Andes hantavirus, spread in South America, is the only known hantavirus with evidence of person-to-person transmission. We detected PUUV-RNA in saliva from NE-patients. However, the patient saliva did not induce seroconversion in inoculated bank voles, indicating that it did not contain infectious virus. This might be due to the fact that human saliva, and especially the salivary component mucin, was able to partly inactivate hantavirus.

LIST OF PUBLICATIONS

I. Jonas Hardestam, Malin Karlsson, Kerstin Falk, Gert Olsson, Jonas Klingström and Åke Lundkvist.
Puumala hantavirus excretion kinetics in bank voles (*Myodes glareolus*)
Emerging Infectious Diseases, Accepted

II. Jonas Hardestam, Melinda Simon, Kjell-Olof Hedlund, Antti Vaheri, Jonas Klingström and Åke Lundkvist
***Ex vivo* stability of the rodent-borne Hantaan virus in comparison to that of arthropod-borne members of the *Bunyaviridae* family.**
Applied and Environmental Microbiology. 2007, 73: 2547-51. PMID: 17337567

III. Jonas Hardestam, Jonas Klingström, Karin Mattsson and Åke Lundkvist
HFRS causing hantaviruses do not induce apoptosis in confluent Vero E6 and A-549 cells.
Journal of Medical Virology. 2005, 76: 234-240. PMID: 15834879

IV. Jonas Klingström, Jonas Hardestam, Malin Stoltz, Bartek Zuber, Åke Lundkvist, Stig Linder, and Clas Ahlm
Loss of cell membrane integrity in Puumala hantavirus-infected patients correlates with levels of epithelial cell apoptosis and perforin.
Journal of Virology. 2006, 80: 8279-8282. PMID: 16873286

V. Lisa Pettersson, Jonas Klingström, Jonas Hardestam, Åke Lundkvist, Clas Ahlm och Magnus Evander**.**
Hantavirus RNA in saliva from patients with hemorrhagic fever with renal syndrome.
Emerging Infectious Diseases. 2008, 14: 406-411. PMID: 18325254

VI. Jonas Hardestam, Lisa Pettersson, Clas Ahlm, Magnus Evander, Åke Lundkvist and Jonas Klingström.
Antiviral effect of human saliva against hantavirus.
Submitted

CONTENTS

LIST OF ABBREVIATIONS

ANDV	Andes virus
CCHFV	Crimean Congo hemorrhagic fever virus
CD	cluster of differentiation
CK 18	cytokeratin 18
CPE	cytopathic effect
cRNA	complementary RNA
CTL	cytotoxic T-lymphocyte
DAF	decay-accelerating factor
DISC	death inducing signalling complex
DNA	deoxyribonucleic acid
DOBV	Dobrava virus
EBV	Epstain-Barr virus
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
ER	endoplasmic reticulum
FADD	Fas-associated death domain
Gc	carboxyl-terminal part of glycoprotein precursor
Gn	amino-terminal part of glycoprotein precursor
gp	glycoprotein
GPC	glycoprotein precursor
GrB	granzyme B
HCPS	hantavirus cardiopulmonary syndrome
HEK	human embryonic kidney
HFRS	hemorrhagic fever with renal syndrome
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HUVEC	human umbilical vein endothelial cells
ICAD	inhibitor of caspase activated deoxyribonuclease
IFN	interferon
Ig	immunoglobulin
IL	interleukin
im	intramuscular
in	intranasal
ip	intraperitoneal
LDH	lactate dehydrogenase
mRNA	messenger RNA
MxA	myxovirus resistance A
NE	nephropathia epidemica
NK	natural killer
NO	nitric oxide
NYV	New York virus
PAMP	pathogen-associated molecular pattern
PARP	poly (ADP-ribose) polymerase
PHV	Prospect Hill virus

PRR	pattern recognition receptor
PUUV	Puumala virus
RdRp	RNA-dependent RNA-polymerase
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
S, M, L-segment	small, medium, large segment
SAAV	Saaremaa virus
sc	subcutaneous
SEOV	Seoul virus
SFSV	Sandfly fever Sicilian virus
SLPI	secretory leucocyte protease inhibitor
SNV	Sin Nombre virus
TNF	tumor necrosis factor
TNF-R	tumor necrosis factor receptor
TRADD	TNF receptor-associated death domain
TRAIL	TNF-related apoptosis inducing ligand
TRAIL-R	TNF-related apoptosis inducing ligand receptor
TULV	Tula virus
TUNEL	terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling
UV	ultraviolet
VEGF	vascular endothelial growth factor
vRNA	viral RNA

For a complete list of hantavirus abbreviations see table 1.

1 HANTAVIRUS BACKGROUND

The hantavirus genus within the *Bunyaviridae* family is a group of viruses that are spread and maintained worldwide in different rodent and insectivore species. Infection of humans is believed to mainly be caused by inhalation of aerosolized contaminated rodent excreta. Disease in humans can have a large variation of severity, from asymptomatic to death, partly depending on the hantavirus that causes the infection.

1.1 THE DISCOVERY OF A NEW ZOONOTIC AGENT

Infectious diseases are often known long before the discovery of the microbiological agents that cause them. This is the case also for hantaviruses. More than 1000 years ago, Chinese physicians described a disease with specific symptoms similar to what is today known as hemorrhagic fever with renal syndrome (HFRS) [1]. Similar diseases with names like epidemic hemorrhagic fever and Songo fever caught attention during the 20th century, often in association with human conflicts, e.g. the "trench nephritis" that affected many soldiers during World War I [2]. During the Korean War, 1950 – 1953, more than 3000 American soldiers fell ill and hundreds died in a similar disease, also this time associated with kidney failure and, sometimes, severe hemorrhagic manifestations [3]. The disease was named Korean hemorrhagic fever. A contemporary report demonstrates the uncertainty about the natural source of the infectious agent:

> "*Evidence is strong that it is an infectious disease (caused by a not as yet identified agent) and one which is probably not transmitted directly from person-to-person but rather a disease in which exposure in man results from contact with some arthropod vector which harbours the etiologic agent.*" [4].

The discovery of this unknown agent did not come about until 1976 when Ho Wang Lee and colleagues identified the virus causing the disease that had been known for so long time [5]. The virus was isolated from a striped field mouse (*Apodemus agrarius*) captured in a village called Songnaeri and was named Hantaan virus after the river running nearby [3]. A few years later the newly discovered virus was successfully propagated in the human carcinogenic lung epithelial cell-line A-549, and electron microscopy (EM) revealed that it represented a new genus within the *Bunyaviridae* family [6, 7]. This was quite surprising, since all other genera of this virus family were strictly arthropod-borne.

1.2 THE HANTAVIRUSES

Shortly after the discovery of HTNV, another pathogenic Korean hantavirus was identified and named Seoul virus (SEOV). The rodent host in this case was the brown rat (*Rattus norvegicus*) [8]. During 1980, the first hantavirus was found in Scandinavia [9]. It was identified by antigen detection in the lungs of a bank vole that had been caught in Southeast Finland and the virus was isolated shortly afterwards by passage in colonized bank voles [10]. The virus was named Puumala virus (PUUV), also this time after the area where the rodent was trapped. PUUV appeared to be the causative agent of nephropathia epidemica (NE), a milder variant of HFRS [11, 12], which was first described independently by two Swedish physicians in 1934 [13, 14]. A strain different from the Finnish PUUV prototype has since then been isolated from a patient in Umeå, Sweden [15]. In Year 1993, a new hantavirus was isolated from a striped field mouse

captured in Slovenia, and named Dobrava virus (DOBV)[16]. However, not until 1995, when a number of soldiers contracted severe HFRS during the conflicts in former Yugoslavia, was DOBV proven pathogenic for man [17].

The first hantavirus reported from the Americas was Prospect Hill virus (PHV), identified in 1982 [18, 19]. It appears to be non-pathogenic to humans, and for about a decade this was thought to be a major discrepancy between hantaviruses in Europe/Asia and the Americas. Unfortunately, this was soon proven wrong. In 1993, a mysterious pulmonary disease started to spread in the Four Corner states (New Mexico, Colorado, Utah, and Arizona) of the southwestern United States. Patients suddenly fell ill and many died shortly after due to shock or pulmonary edema. These symptoms had earlier not been associated with hantaviruses. Intensive laboratory work revealed that the causing agent indeed was a hantavirus, the first pathogenic one to be discovered in the Americas. After intense arguments about what to call this new virus, it was finally decided to simply call it Sin Nombre virus (SNV) [20].

After an outbreak of severe pulmonary syndrome among patients in Argentina in 1996, yet another novel hantavirus was isolated, this time from the long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*), and named Andes virus (ANDV). Since then there has been an explosion of hantavirus discoveries in South America, with more than 15 new variants reported so far (Fig. 1).

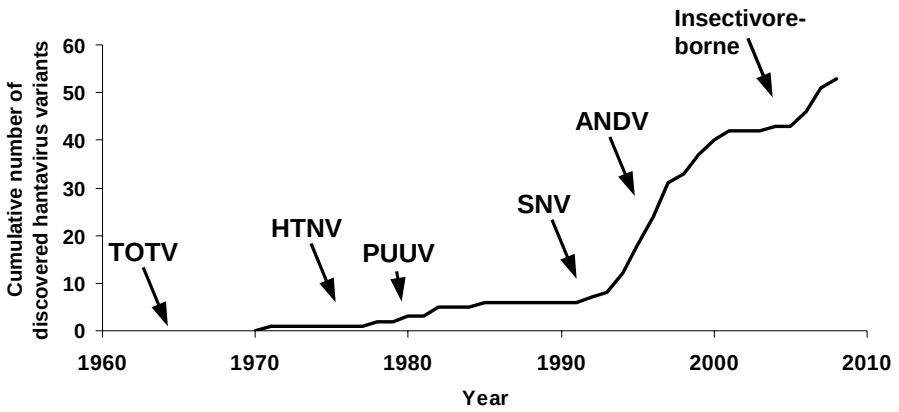

Figure 1. Cumulative number of hantavirus variants known so far.

To date, about 54 different hantavirus variants have been identified worldwide (Table 1). Some of these represent officially recognized novel hantaviruses, whereas others are new strains of already known viruses. Another subset so far only represents discoveries of a novel hantavirus sequence. Most hantaviruses are associated with a specific rodent or insectivore host, or a few closely related species. The remarkable similarity between the evolutionary trees of the hantaviruses and their rodent hosts indicates millions of years of co-evolution [21], probably since the common ancestor of the three rodent (*muridae*) subfamilies, *Murinae*, *Sigmodontinae* and *Arvicolinae*, about 50 million

years ago [22] (Fig. 2). Interestingly, the association of particular hantaviruses to their host animals of the three different rodent subfamilies appears to have caused differences in the ability to cause disease in humans [23]. The *Murinae* subfamily seems to mainly carry hantaviruses that cause severe HFRS, whereas the *Sigmodontinae*-borne hantaviruses cause hantavirus cardiopulmonary syndrome (HCPS). The *Arvicolinae* subfamily is instead associated with hantaviruses causing mild HFRS, like NE, or no disease at all.

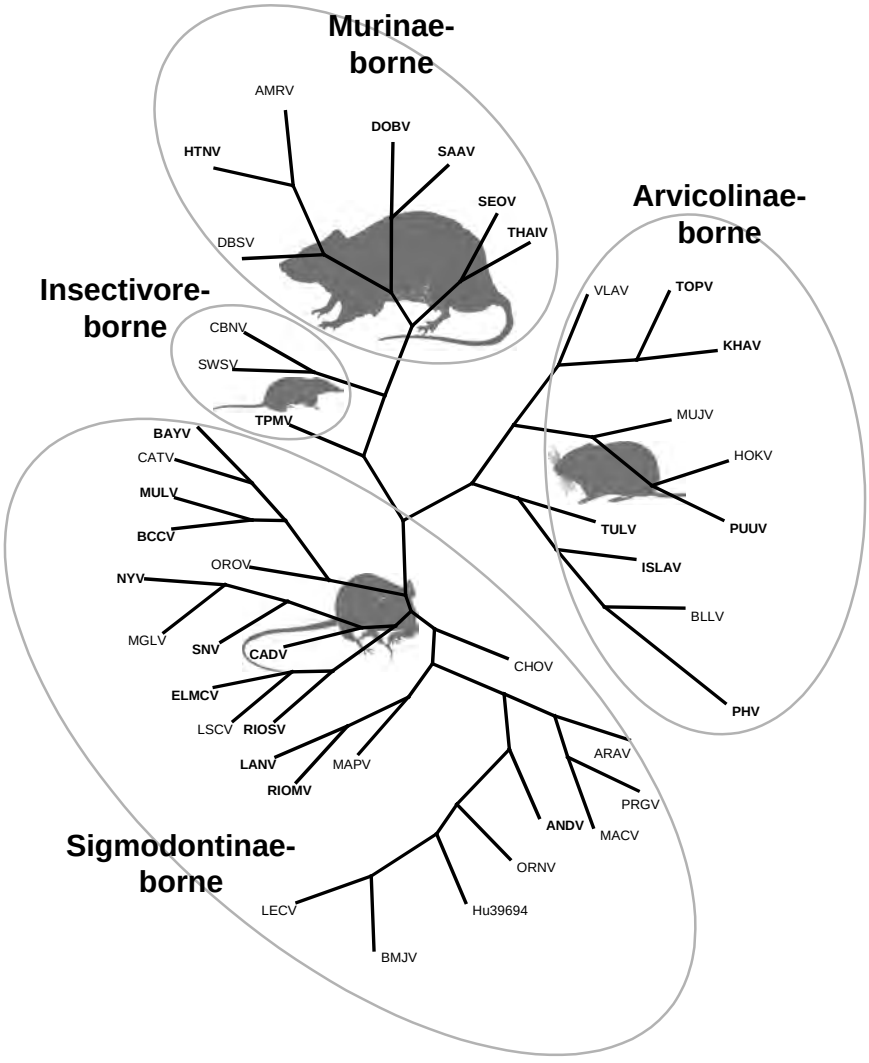

Figure 2. Phylogenetic tree of hantavirus variants. For abbreviations see table 1. Officially recognized virus species are shown in bold. Kindly provided by Dr. Kirill Nemirov.

Almost all hantaviruses have so far been isolated from rodents in Europe, Asia and the Americas, but increasing evidence is now indicating that hantaviruses are also present in other parts of the world. It is probably just a matter of looking carefully enough. The occurrence of anti hantavirus antibodies among humans in Africa have been known for several years [24] and in 2006, hantavirus genome sequences were detected in an African wood mouse (*Hylomyscus simus*) captured in Guinea, indicating that hantaviruses are present also on this continent [25]. Since then, a novel sequence of hantavirus RNA has been recovered from the Therese's Shrew (*Crocidura theresae)* also caught in Guinea [26]. This finding together with the recent isolation of hantaviruses from different species of shrews in the USA, Switzerland and Vietnam is interesting [27-30], since for a long time the only hantavirus known to be associated with an insectivore host was the Thottapalayam virus (TOTV), which was isolated from a Musk shrew in India in 1964 [31]. If the occurrence of TOTV and the other hantaviruses found in shrews are not explained by a more recent host switch, there is reason to believe that co-evolution has occurred since the divergence of rodentia and insectivore, about 100 million years ago [32, 33]. Interestingly, the rate of molecular evolution in the hantaviruses has recently been estimated to be much faster than the earlier assumptions, and a re-evaluation of the co-divergence hypothesis has been suggested [34].

As the insectivore-borne hantaviruses known so far still constitute a minority within the genus and show no evidence of human infections, hantaviruses will in this thesis be described simply as rodent-borne viruses.

Table 1. Hantavirus variants (officially recognized virus species are shown in bold).

Virus (Abbr.)	Disease	Rodent host	Originally discovered in	Ref.
Old World Hantaviruses				
Murinae subfamily-associated viruses				
Hantaan (HTNV)	HFRS	Striped field mouse (*Apodemus agrarius*)	Korea	[5]
Amur (AMVR)	HFRS	Korean field mouse (*Apodemus peninsulae*)	Russia	[35]
Da Bie Shan (DBSV)		Chinese white-bellied rat (*Nivicenter confucianus*)	China	[36]
Dobrava (DOBV)	HFRS	Yellow-necked field mouse (*Apodemus flavicolis*)	Slovenia	[16, 37]
Seoul (SEOV)	HFRS	Black rat (*Rattus rattus*) Brown rat (*R. norvegicus*)	Korea	[8]
Thailand (THAIV)		Bandicoot rat (*Bandicota indica*)	Thailand	[38]
Saaremaa (SAAV)	HFRS	Striped field mouse (*Apodemus agrarius*)	Estonia	[39]
Sangassou (SANGV)		African wood mouse (*Hylomyscus simus*)	Guinea	[25]
Arvicolinae subfamily-associated viruses				
Hokkaido (HOKV)		Red bank vole (*Myodes rufocanus*)	Japan	[40]
Muju (MUJV)	HFRS	Royal vole (*Myodes regulus*)	Korea	[41]
Puumala (PUUV)	HFRS	Bank vole (*Myodes glareolus*)	Finland	[9]
Khabarovsk (KHAV)		Reed vole (*Microtus fortis*)	Russia	[42]
Tula (TULV)	HFRS	European common vole (*Microtus arvalis*)	Russia	[43, 44]
Topografov (TOPV)		Lemming (*Lemmus sibericus*)	Russia	[45]
Vladivostok (VLAV)		Reed vole (*Microtus fortis*)	Russia	[46]
Insectivore-associated viruses				
Cao Bang **(**CBNV)		Chinese mole shrew (*Anourosorex squamipes*)	Vietnam	[29]
Seewis (SWSV)		Eurasian common shrew (*Sorex araneus*)	Switzerland	[28]
Tanganya (TGNV)		Therese's Shrew (*Crocidura theresae)*	Guinea	[26]
Thottapalayam (TPMV)		Musk shrew (*Suncus murinus*)	India	[31]

New World Hantaviruses

Arvicolinae subfamily-associated viruses				
Bloodland Lake (BLLV)		Prairie vole (*Microtus ochrogaster*)	USA, Canada	[47]
Isla Vista (ISLAV)		California vole (*Microtus californicus*)	USA	[48]
Prospect Hill (PHV)		Meadow vole (*Microtus pennsylvanicus*)	USA, Canada	[18, 19]
Sigmodontinae subfamily-associated viruses				
Andes (ANDV)	HCPS	Long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*)	Argentina	[49, 50]
Araraquara (ARAV)	HCPS	Hairy-tailed Bolo mouse *(Bolomys lasiurus)*	Brazil	[51, 52]
Bayou (BAYV)	HCPS	Rice rat (*Oryzomys palustris*)	USA	[53]
Bermejo (BMJV)	HCPS	Chacoan pygmy rice rat *(Oryzomys chacoensis)*	Argentina	[54, 55]
Black Creek Canal (BCCV)	HCPS	Cotton rat (*Sigmodon hispidus*)	USA	[56, 57]
Blue river (BRV)		White-footed mouse (*Peromyscus leucopus*)	USA	[58]
Calabazo	HCPS	Cane mice (*Zygodontomys brevicauda*)	Panama	[59]
Caño Delgadito (CADV)		Alston's cotton rat (*Sigmodon alstoni*)	Venezuela	[60]
Castelo dos Sonhos (CASV)	HCPS	Unknown	Brazil	[51]
Catacamas (CATV)		Coues' oryzomys (*Oryzomys couesi*)	Honduras	[61]
Choclo (CHOV)	HCPS	Pygmy rice rat (*Oligoryzomy fulvescens*)	Panama	[59]
El Moro Canyon (ELMCV)		Western harvest mouse (*Reithrodontomys megalotis*)	USA	[62]
Hu39694	HCPS	Long-tailed mouse *(Oligoryzomys flavescens)*	Argentina	[55, 63]
IP37/38		Black-footed Pygmy Rice Rat *(Oligoryzomys nigripes)*	Paraguay	[64]
Juquitiba (JUQV)	HCPS	Black-footed pigmy rice rat (*Oligoryzomys nigripes*)	Brazil	[52, 65]
Laguna Negra (LANV)	HCPS	Veser mouse (*Calomys laucha*)	Paraguay	[66, 67]
Lechiguanas (LECV)	HCPS	Rice rat (*Oligoryzomys flavescens*)	Argentina	[55]
Limestone Canyon (LSCV)		Brush mouse (*Peromyscus boylii*)	USA	[68]
Maciel (MACV)		Dark field mouse (*Necromys benefactus*)	Argentina	[55]

Maporal (MAPV)		Pygmy rice rat (*Oligoryzomus fulvescens*)	Venezuela	[69, 70]
Monongahela (MGLV)	HCPS	Deer mouse (*Peromyscus maniculatis*)	USA	[71]
Muleshoe (MULV)		Hispid cotton rat (*Sigmodon hispidus*)	USA	[72]
New York (NYV)	HCPS	White-footed mouse (*Peromyscus leucopus*)	USA	[73, 74]
Oran (ORNV)	HCPS	Long-tailed Pygmy Rice Rat (*Oligoryzomys longicaudatus*)	Argentina	[54]
Pergamino (PRGV)		Grass field mouse (*Akodon azarae*)	Argentina	[55]
Playa de oro (OROV)		Coues' Rice Rat *(Oryzomys coues),* Jaliscan Cotton Rat *(Sigmodon mascotensis)*	Mexico	[75]
Rio Mamore (RIOMV)		Small-eared pygmy rice rat (*Oligoryzomys microtis*)	Bolivia	[76, 77]
Rio Segundo (RIOSV)		Mexican harvest mouse (*Reithrodontomys mexicanus*)	Costa Rica	[78]
Sin Nombre (SNV)	HCPS	Deer mouse (*Peromyscus maniculatis*)	USA	[20, 79]
<u>*Insectivore*-associated viruses</u>				
Camp Ripley (RPLV)		Northern short-tailed shrew (*Blarina brevicauda*)	USA	[30]
Ash River (ARRV)		Masked shrew (*Sorex cinereus*)	USA	[27]
Jemez Springs (JMSV)		Dusky shrew (*Sorex monticolus*)	USA	[27]

1.2.1 Hantavirus in Sweden

PUUV, the only known hantavirus circulating in Sweden, is mainly spread north of the Limes Norrlandicus at around 59º-60º N, that among other things constitutes a border between different vegetation types, e.g. the southern mixed deciduous and the northern coniferous forest. The number of NE patients fluctuates yearly and reflects the cyclic variation in the density of the natural host, bank vole (*Myodes glareolus*) (Fig. 3), that peaks every three to four years [80-83]. Why infected bank voles (and thereby humans) are found almost solely in the northern part of Sweden, despite the fact that bank voles are endemic throughout the country, remains unknown.

Figure 3. Bank vole (*Myodes glareolus*), the natural host of Puumala virus. This photograph was kindly provided by Jens Schou, Biopix.

In 2007 as many as 2195 cases of NE were reported in Sweden, which was the highest number since the disease became notifiable in 1989, and almost four times higher than the previous record from 1998 with 562 reported cases [84]. During 2007, as many as 313 patients/100,000 persons in the Västerbotten county was reported, as compared to the median incidence of 38 for the same area during the last 10 years [84]. This outbreak was recently investigated by Petterson and colleagues, who found that there were fewer days with ground snow cover in December 2006 compared to December the two previous bank vole peak periods (2001-2002 and 2004-2005) [85]. Since snow cover provides an important protection for small rodents, this might have contributed to an increased bank vole infestation of human dwellings and thereby to an increased risk

for human exposure [85]. Interestingly, before this outbreak, bank vole numbers in autumn 2007 (and consequently the number of human cases) were predicted, and subsequently verified by trapping, to be the highest since the early 1970´s (Olsson G. et al., submitted manuscript).

1.3 STRUCTURE AND REPLICATION

Hantaviruses particles are spherical or ovoid and 80-210 nm in diameter [86] (Fig. 4). The virions are enveloped and contain a single stranded, negative sense, tri-segmented RNA-genome, which consist of the small (S), medium (M) and large (L) segments, encoding the nucleocapsid protein (N), the glycoprotein precursor (GPC) of two glycoproteins (Gn and Gc), and the RNA-dependent RNA-polymerase (RdRp), respectively. In addition to the structural proteins, the S segments of PUUV and Tula virus (TULV) have recently been shown to also encode a non-structural protein, which seems to down-regulate the host interferon (IFN) response [87].

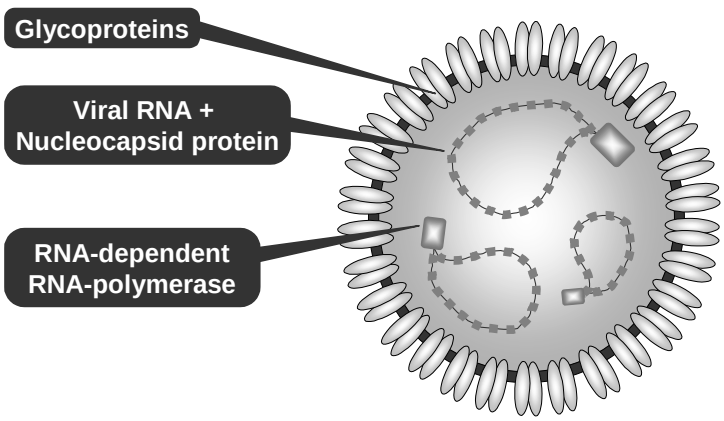

Figure 4. Hantavirus particle.

Infection starts by the binding of hantavirus Gn and Gc to integrin receptors of the human cell [88, 89] (Fig. 5). Decay-accelerating factor (DAF) has recently been suggested as a critical co-factor for entry [90]. The binding is followed by receptor-mediated endocytosis via the clathrin-coated pit pathway [91]. Once inside the cell, the endo-lysosome and the viral membrane are fused and the viral genome enters the cytoplasm. Like all other negative stranded RNA viruses, hantaviruses carry their own RdRp into the cell where it immediately starts the transcription of messenger RNA (mRNA). The primers needed for viral transcription are aquired from the host cell mRNAs through a process called cap-snatching [92, 93], which was first described for influenza virus [94]. Soon after the initial transcription, synthesis of antigenomic complementary RNA (cRNA) is started. The initiation of both transcription and replication has been suggested to follow the “prime-and-realign” principle [93]. The produced cRNA will in turn be used as template for production of new viral RNA (vRNA). The produced vRNA can serve as an additional template for mRNA or, at later stages, as the genome of progeny virions [95]. Like for any cellular protein, the translation of viral S, M and L mRNA takes place on ribosomes.

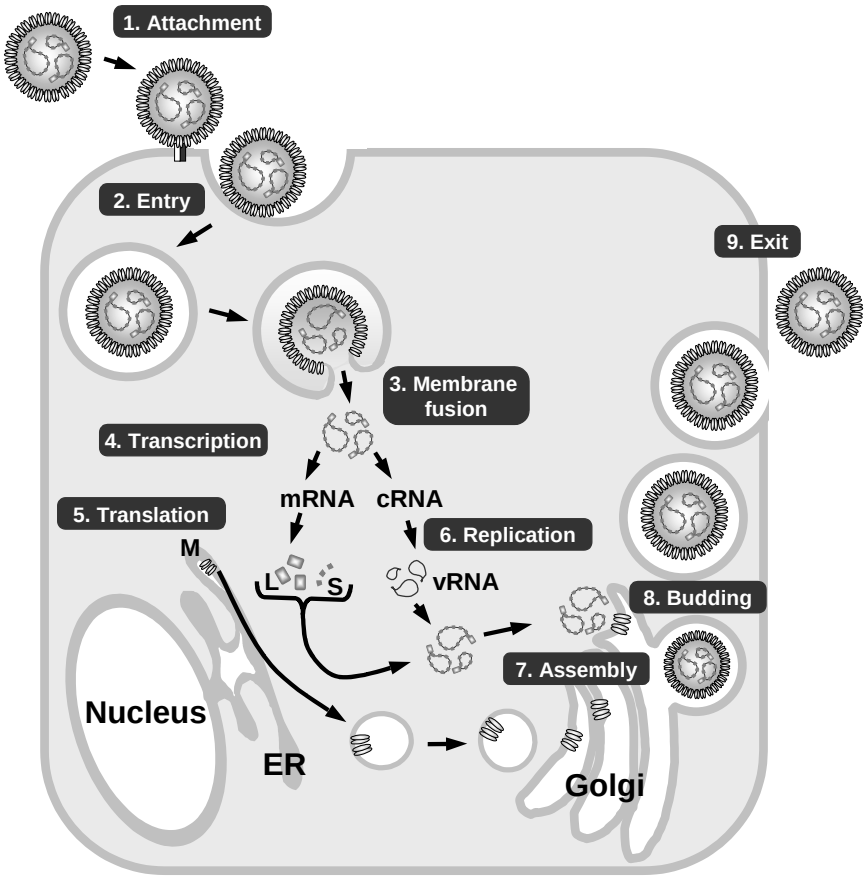

Figure 5. Hantavirus replication.

L and S segment mRNA is translated on free ribosomes to generate the RdRp and the N, respectively. The N is synthesized first and is accumulated early after infection. M segment mRNA is translated on membrane bound ribosomes to generate the GPC, which is cleaved to Gn and Gc at the conserved WAASA-motif [96]. After addition of N-linked oligosaccharides, the Gn and Gc proteins dimerize in the endoplasmic reticulum (ER) [97]. Interaction of the Gn and Gc in the ER seems to be crucial in order for them to be transported to the Golgi complex [98]. Glycoproteins are transported in vesicles through yet unknown mechanisms to the Golgi, where they accumulate [99]. Retention of the glycoproteins at the Golgi complex is thought to be responsible for the maturation of hantaviruses at the Golgi, where assembly takes place [95, 100]. Unlike many other negative stranded RNA viruses, hantaviruses do not have a matrix protein. Therefore it is likely that the cytoplasmic tails of the Gn and Gc directly interact with the N during assembly [95]. Unlike most other enveloped viruses, the members of the *Bunyaviridae* family obtain their membrane by budding from the Golgi and are thereafter transported in vacuoles to the plasma membrane where they are released by

exocytosis [101]. In contrast to Golgi maturation, EM studies of SNV and Black Creek Canal virus have suggested the cell surface as an alternative maturation and budding site for the New world hantaviruses [86, 102].

1.4 CLINICAL SYNDROMES

Hantaviruses cause two distinct diseases in humans, HFRS in Europe and Asia and HCPS in the Americas. HFRS is characterized by fever, hemorrhagic manifestations and renal impairment, whereas HCPS is associated with cardiopulmonary dysfunction. It should however be pointed out that some HFRS-patients show symptoms of pulmonary dysfunction and some HCPS-patients have renal dysfunction [103]. Irrespective of the clinical manifestations, both diseases involve the same principal abnormality, a vascular dysfunction [104].

1.4.1 HFRS

About 150,000 hospitalized HFRS-patients are recognized each year, predominantly in China, but also in other parts of Asia and in Europe [2]. After inhalation of aerosolized virus, infection starts in the human lungs. The incubation period is typically 2-3 weeks but can be as long as 42 days [105]. After infection of primary target cells, i.e. endothelial cells in the lung [106], virus is spread via the blood to other organs. Typically, HFRS can be divided into five phases: the febrile, hypotensive, oliguric, polyuric and convalescent phases. The febrile phase, lasting 3-5 days, starts with an abrupt onset of high fever which soon is followed by headache, nausea, abdominal pain, backache and thirst [105, 107]. Some HFRS-patients show conjunctival hemorrhage and fine petechiae, often together with proteinuria [105]. The disease progresses into the hypotensive phase, which can lead to cardiovascular collapse and severe or lethal shock. The thirst, abdominal pain and the decreased blood pressure are all manifestations of the increased capillary permeability. The hypotensive phase lasts from several hours up to two days and is followed by the oliguric phase, which lasts from a few days to two weeks during which hemorrhages continues and anuria can occur. Subsequently renal function returns, which marks the beginning of the polyuric phase. Although this phase is a positive sign for the patient [104] it also involves new problems in terms of fluid and electrolyte management [108]. The convalescent phase can last for several weeks or months before full recovery is reached.

NE, caused by PUUV, is a milder form of HFRS [12] and normally displays a less distinct pattern of the five phases observed for HFRS. The febrile phase of NE is generally less severe, shock seldom occurs and hemorrhages are generally limited to petechiea [11]. Also the renal failure is often less severe and sometimes absent. Up to one third of the patients experience blurred vision [11]. Due to these vague symptoms, NE is often misdiagnosed [108]. Deaths due to NE are rare and the case fatality rate is 0.1-1% [23, 109]. The proportion of subclinical cases is large and seroprevalence has been reported to be as high as 8.9% in northern Sweden [110].

1.4.2 HCPS

About 500 cases of HCPS have been reported in the United States since the disease was first recognized in May 1993 [111]. After an incubation period of 1-5 weeks [112]

HCPS typically progresses through four different phases: febrile, cardiopulmonary, diuretic and convalescence. The febrile phase (3-5 days) is in many ways similar to that described for HFRS and for many other viral prodromes. A dry cough can appear at the end of this phase due to the impending pulmonary edema. The cardiopulmonary phase is characterized by severe pulmonary edema, hypotension, oliguria and shock. Once pulmonary edema is present, patients may die within 24-48 hours [113]. In surviving patients, the disease proceeds to the diuretic phase, which is characterized by a rapid clearance of the pulmonary edema. The shock and fever are resolved and a convalescent phase of up to 2 months follows before the patient fully recovers.

1.5 TREATMENT, PREVENTION AND CONTROL

1.5.1 Antiviral drugs

Ribavirin is the only antiviral drug in use against hantavirus infections. An anti-viral effect *in vitro* [114] and in suckling mice [115] has been observed. The drug has also been tested on HFRS-patients in a double blind trial where the ribavirin-treated group showed a seven-fold reduction in morbidity as compared to the placebo control [116]. However, for the treatment of HCPS, ribavirin seems not to be effective [117].

1.5.2 Vaccines

In light of the worldwide distribution of hantaviruses, the severity of many hantavirus infections, and the lack of effective treatment, it is easy to see the need for an effective and easily produced vaccine. Several attempts to develop a hantavirus vaccine, such as recombinant live vaccines, virus-like particles, naked DNA and recombinant proteins, have showed promising results *in vivo*. However, the only commercially available candidate so far is Hantavax™ [118], which has been used extensively in China and Korea. The use of Hantavax™ has been connected to a 45% decrease in Korean HFRS-cases between 1991 and 1996 [119]. Although Hantavax™ seems to be well tolerated in humans [120], the production by formalin inactivating HTNV grown in rat or mouse brains is a controversial technique that has precluded its use in many countries [121]. Cell culture derived inactivated vaccines, developed and tested in human trials in China, have been shown to produce effective immunity with high levels of neutralizing antibodies and fewer side effects compared to the rodent-brain derived vaccine [122, 123]. However the long-term efficacy of both the rodent brain and the cell-line derived vaccines has been questioned as levels of neutralizing antibodies are quite low as soon as one year after vaccination and frequent boosters seem to be needed [104, 121, 124].

1.5.3 Passive immunization

To date, no specific treatment or pharmacologic prophylaxis is available for HFRS or HCPS. A strategy for therapeutics or for a short-term instant prevention of infection can instead be the passive transfer of antibodies. During the acute phase of HCPS, a strong correlation between a positive outcome of the disease and the patients' titers of neutralizing antibodies has been observed, which suggests that a strong serologic response is important during infection [125, 126]. So far no studies of passive immunization in man have been reported, but two promising animal studies have been performed recently. Cynomolgus macaques showed sterile protection against PUUV challenge after being passively immunized with sera from previously infected monkeys

[127] and passive transfer of neutralizing antibodies produced by DNA vaccination of rabbits, protected Syrian hamsters from lethal intranasal challenge of ANDV [128].

1.5.4 Control

Most hantavirus transmissions to humans are believed to occur inside or close to man-made structures and rodent-proofing houses previously infested by rodents has been shown to decrease the risk of infection substantially [129]. Hantaviruses are easily inactivated by detergents, chlorine solutions or other house-hold disinfectants [130, 131] and when removing rodent excreta, wet wiping is preferable to vacuum cleaning in order to avoid production of aerosols [131]. An increased awareness among physicians for the sometimes rather indefinable symptoms of HFRS and HCPS is also desired, as early supportive treatment is crucial.

2 TRANSMISSION

Unlike all other genera of the *Bunyaviridae* family, the hantaviruses are non-vector-borne viruses, and are transmitted either directly via contacts or indirectly via the environment. Hantaviruses have therefore been suggested to be called roboviruses (rodent-borne) as compared to the term arboviruses (arthropod-borne) used for the other genera within the *Bunyaviridae* family [21]. By investigating the mechanisms by which hantaviruses are moving from rodent to rodent and identifying the factors that influence the transmission, being viral, host or environmental factors, more sophisticated models for hantavirus transmission can be constructed and used to predict outbreaks and prevent the transmission of hantavirus to humans. Hantaviruses are forced to adopt different strategies in order to maintain their circulation in the seasonally often highly variable rodent population. Such strategies may include persistent infection of the host, massive and prolonged virus shedding and extended *ex vivo* stability of the virions.

Various reports have shown the occurrence of anti-hantavirus antibodies in several wild animals, e.g. hares, deer, owl, moose, as well as domestic animal like cows, cats and dogs [132, 133] and limited data has shown the excretion of infectious hantavirus from pigs [134]. However, before the occurrence of excreted infectious virus or at least viral RNA from these animals is further studied, the significance for the transmission to humans is still unclear.

2.1 BETWEEN HOSTS AND FROM HOST TO HUMAN

The higher the number of infected rodents, the higher the risk for human transmission, which means that many factors that influence hantavirus transmission between rodents indirectly also influence the risk for humans to get infected [80]. There are also common factors, e.g. temperature or humidity in the environment, which by affecting the *ex-vivo* stability of the virus, directly influences both rodent-to-rodent and rodent-to-human transmission.

2.1.1 Host factors

2.1.1.1 Behavior

Host behavior can to a large extent influence the transmission rate of a pathogen [135]. In fact, there are several examples of pathogens that induce a behavioral change of the infected host, in order to increase fitness benefits for the pathogen [135-137]. If a pathogen is transmitted through social contact, natural selection should favor those pathogens that induce behaviors that lead to an increased amount of interactions with other animals [138]. Studying behavior in wild animals has its obvious difficulties. One way by which aggressive behavior among rodents can be studied retrospectively is by the appearance of wounds, if unbiased from other influencing factors like age and sex. In laboratory experiments, SEOV has been shown to be more effectively transmitted via intramuscular injections than by inhalation of aerosols [139] and a correlation between wound appearance and hantavirus infection of wild rodents has been observed for SEOV and SNV [140-143]. These findings suggest that biting is the main route for transmission of these viruses, and that the virus therefore would benefit from a more

aggressive behavior of the host. If biting is not the main route, an alternative explanation might be that more urine aerosol is produced during fights, thereby favoring the transmission of virus via urine [144]. It is however hard to distinguish if hantaviruses cause changes in certain behaviors or if an animal's behavior affects its likelihood of getting infected. It is also possible that the correlation between aggressive behavior and hantavirus infection is due to the fact that aggressive rodents, apart from fighting, also are more engaged in mating, or are more active in general [145, 146].

Laboratory experiments have shown that SEOV infection of male adult rats did indeed increase aggressive behavior and further, that while the more aggressive rats had more virus present in their organs, they did not shed more virus in saliva, urine or feces [138]. Experimental infection with PUUV was shown to induce increased urination frequency in bank voles (De Jaegere F. and Lundkvist Å., unpublished data). The mechanisms behind these phenomena is not clear, but the induced change in behavior is suggested to affect the brain indirectly via the action of hormones rather than by direct effects [145], since hantavirus has been shown to infect the testes [141] but rarely crosses the blood brain barrier [141, 147].

In contrast, although ANDV RNA was only found in saliva, a correlation between wounding and infection rate was not found when studying ANDV transmission in caged wild pygmy rice rats (*Oligoryzomys longicaudatus*) and it was therefore speculated that virus in saliva is transmitted via other routes than biting, such as food contamination or production of saliva aerosols [148].

2.1.1.2 Sex

Males of many species are, compared to females, more susceptible to infectious diseases [149] and this is the case also for the hantavirus hosts [143]. The difference appears after sexual maturation and therefore a change in hormone production has been hypothesized to cause the difference between the sexes [143]. Hormones might either change male behavior, and thereby increase their exposure to the virus, or alternatively, hormones regulate the immune response, thereby rendering the males susceptible to the infection [150, 151], or a combination of both. When adult rats where experimentally inoculated with SEOV, both sexes where equally susceptible to the infection whereas males shed virus for longer time and via more routes, and also had higher levels of viral RNA in their blood [150, 152]. One factor that can explain the higher seroprevalence among males is that they are more often engaged in fights [153]. Another factor could be that the larger home range of males leads to an increased risk for transmission by aerosols [154].

2.1.1.3 Age

Hantavirus infections in general do not seem to influence the age of the animal and infection of adult animals is chronic and non-pathogenic [155, 156]. However, it has been shown that PUUV infected bank voles have a lower over-winter survival probability than antibody negative bank voles, suggesting that infection has a negative effect on the voles [157]. Whether this is caused by the infection *per se*, or an underlying factor e.g. more risk prone behaviour, resulting in early infection and death, remains unknown and is probably difficult to elucidate. In wild caught rodents, weight

(used as an indicator of age) strongly correlates with the prevalence of hantavirus infection [82, 142, 155, 156, 158], which indicates a horizontal transmission route [144]. For many hantaviruses a correlation has been observed between the likelihood of the respective host rodent being infected and reaching the age of physiological maturity [140, 159, 160]. The increased seroprevalence observed among young animals is explained by the transfer of antibodies from the mother to pups via the placenta and during lactation [161, 162]. Passive immunization with maternal antibodies has been reported to protect the pups for 3-3.5 months [159] or up to 145 days [163].

2.1.1.4 Immune defense – viral persistence

Hantavirus infections in humans are transient and cleared by the immune response whereas the infection in the natural host is generally believed to be persistent. The reason behind virus persistence may be that hantaviruses either down-regulate or evade host immune responses or due to the ability of hantaviruses at some point after infection to down-regulate replication or induce antigenic variation [144]. Regulatory T-cells have been suggested to mediate SEOV persistence in Norway rats [164] and SNV persistence in deer mice [165].

The association of hantavirus to the brown fat tissue of the natural host has been suggested to be important for the maintenance [147, 166]. However, it has been argued that, in nature, viral persistence for a few months is probably enough to encompass the life span of the host [144]. On the other hand, maintenance of infection in only a few long-lived animals might be important for the virus to remain in circulation during periods of low host population density [142].

2.1.2 Host population density

Many rodent populations show cyclic variations in density with recurrent periods of very few animals and the density of the rodent host population is connected to the number of hantavirus infections among humans [80-83]. This effect is simply due to the fact that a higher number of rodents results in a higher absolute number of infected rodents. One example is the increase in rodent populations preceding the outbreak of HCPS in the USA in 1993, which is believed to have been associated with an increase in precipitation, associated with an El Niño southern oscillation and leading to increased food resources [103]. However, whether host seroprevalence is correlated to host density is not completely clear and the appearance of this relation might partly be an effect of the sampling frequency in field studies. According to several studies, the density of the host population is one of the factors that affects the host hantavirus seroprevalence in nature [82, 158, 167]. Host seroprevalence can also be delayed-density-dependent as shown for SNV infection in deer mice, where the seroprevalence during spring is associated to the host density the previous fall [168, 169]. Host population density can also be inversely proportional to the hantavirus seroprevalence as shown by several epidemiological studies in the Americas [156, 170, 171]. This phenomenon might be explained by the fact that high densities of rodents with high reproductive success and high turnover rate, generates populations with a large proportion of young (seronegative) animals. When these populations decrease, seroprevalence increases due to the gradually larger proportion of older (infected) animals [168, 169]. However, although prevalence (calculated as a percentage) is low

during periods of high rodent densities, there can still be a substancial risk for human infections since the actual numbers of infected rodents are higher [170].

2.1.3 Environmental factors

The density of rodent populations are very much affected by variations in environmental factors, such as vegetation type, food supply, predator abundance, protective snow cover and temperature. Remote sensing (satellite photography) together with geographic information systems characterizing areas regarding vegetation type, elevation, slope and hydrologic features have been used to estimate the infection status of deer mice with 80% accuracy, and subsequently also the risk of human infections [172]. Similarly, efforts to correlate landscape characteristics and the occurrence of HFRS have been made in China [173]. Other authors point out the importance of adding variables related to human behaviour and land use (in addition to land cover and habitat variations) in epidemiological models that should predict disease risk for humans [174].

2.1.4 Shedding of virus

For viral transmission efficacy, persistent infection of the host is of little value unless virus is also shed to the environment and thereby to new hosts. For several hantaviruses, the levels of shed virus seem to peak a few weeks after infection and then decrease to low or even undetectable levels [159, 175, 176, paper I] (Table 2).

Table 2. A summary of studies investigating shedding of hantaviruses after experimental infection of natural hosts. Bold letters indicate time points when positive shedding occurred, whereas normal letters indicate a selection of the days when excretions where negative.

	Detection of virus in excretions at days post infection					
Virus (route)	Saliva/oropha-ryngeal secretions	Urine	Feces	Blood / organs	Method for detection	Ref.
PUUV(sc[A])	4, **8-84**, 91	9, **11-70**, 77	9, **11-44**, 49	Blood: **133**	Real time RT-PCR	[Paper I]
PUUV (im[B])	7, **14, 21, 28**, 35	35, **42**, 49, 56, **63**, 99	28, **35, 42, 49, 56, 63, 99, 103**, 191	Blood: 7, **10, 14**, 21 (Antigen in lung d. 270)	Im inoculation of weanling bank voles	[175]
HTNV (in[C])	**7, 10, 15, 20, 25, 30, 40**	**7, 10, 15, 20, 25, 30, 40**	**15, 20, 25**, 30, **40**	Lung: 15, **20, 25, 30, 40**	Im inoculation of Apodemus mice	[177]
HTNV (im)	**9, 12, 15, 25, 35, 45**	**9-360**	**9, 12, 15, 25, 35, 45**	Blood: **7, 9, 12, 15**	Im inoculation of Apodemus mice + intracage transmission d. 10-35	[177]
SEOV (ip[D])	0, **10, 20, 30, 40**	0, **10, 20, 30, 40**	0, **10, 20, 30, 40**	Lung: 0, **10, 20, 30, 40**	Nested RT-PCR	[152]
SNV (im)	8, **15**, 22, 29, **43**, 51, **60, 78, 90**, 120	8, 15, 22, 29, 43, 51, 60, 78, 90, 120	8, 15, 22, 29, 43, 51, 60, 78, 90, 120	Blood: **120, 180**	Real time RT-PCR	[176]

[A]sc, subcutaneously
[B]im, intramuscularly
[C]in, intranasally
[D]ip, intraperitoneally

A variation in the levels of shed virus with periods of absent shedding followed by recurrence of shedding has also been observed in both wild and colonized animals [175, 178, 179, paper I]. Whether the observed variation is induced by stress, immune factors, hormones or some unknown physiological factor or are just due to technical errors of the assays, remains to be shown. Despite numerous reports on the subject [141, 148, 152, 175-177, 180-182, paper I] it is hard to determine a certain route of major importance for shedding of hantaviruses.

2.1.5 Virus *ex vivo* stability

An ability to survive outside the host, which probably is important for the transmission efficacy, has been shown for several viruses such as hepatitis A virus [183, 184], avian influenza virus [185] and astroviruses [186]. The *ex vivo* stability becomes extra important in order to maintain an endemic infection in rodent populations that often undergo strong density fluctuations, which has been highlighted by mathematical modeling based on epidemiological field data for PUUV in bank voles [187, 188]. Environmental factors such as UV-irradiation, temperature, and soil moisture have effects on the *ex vivo* survival of several viruses [189, 190] and a close correlation has been observed by wet habitats and the occurrence of PUUV antibody positive bank voles [155]. The reason for this correlation might be that humidity increases the ability of the excreted virus to survive outside the host. In laboratory experiments the *ex vivo* stability of hantaviruses has been shown to persist much longer in a humid as compared to a dry environment [191, paper II]. It has been suggested that above a certain density of host animals, the risk for human infection is decided by environmental factors that affect the virus stability outside the host (soil moisture etc.) rather than factors that affect the number of host animals (climate, landscape configuration etc.) [192]. Further, it has been shown that bank voles caught on northerly facing slopes and on marsh land more often are infected by PUUV (independent of the bank vole density), suggesting the importance of environmental conditions for virus *ex vivo* survival and transmission rates [155]. The *ex vivo* stability of PUUV has been tested by using colonized bank voles in an intra-cage system. Excreted virus in cage beddings was infectious to naïve recipient bank voles for up to 12-15 days after removal of the infected donors [191]. However the *ex vivo* stability seen for hantaviruses does not seem to be a unique property due to the non vector-borne transmission, as the vector-borne sandfly fever Sicilian virus and the Crimean-Congo hemorrhagic fever virus also showed quite extensive stability in laboratory experiments [paper II].

2.2 HOST TO HUMAN TRANSMISSION

Transmission to humans is believed to occur mainly as a consequence of inhalation of aerosolized virus-contaminated rodent saliva, urine or feces. Other possible routes might be ingestion of contaminated food or water or after direct introduction of virus into broken skin or conjunctiva [131]. There are also a few reports when rodent bites might have caused the transmission [193, 194]. However, when a rodent bites a human, it is probably hard to completely rule out the possibility that aerosolized excreta are also inhaled at the occasion. There are also several reports of laboratory-acquired cases of HFRS [194, 195], many occurring during studies unrelated to hantavirus research, but with unintentionally hantavirus-infected laboratory animals.

Hantavirus infection of humans is epidemiologically related to activities such as entering or cleaning previously vacant rodent-infested human dwellings, cleaning barns and other outbuildings or handling firewood stored in woodsheds and woodpiles [80, 131]. Farmers, mammalogists, construction and forestry workers constitute occupational risk groups for infection in Europe [196-200], whereas there is little or no serological evidence supporting an increased risk among certain occupational groups with exposure to rodents in USA [201-203]. In Sweden, middle-aged men are overrepresented among NE patients [80].

3 IMMUNE RESPONSE AND PATHOGENESIS IN HUMANS

3.1 WHY DO WE GET SICK (AND NOT THE RODENTS)?

The fact that there are hantaviruses that are pathogenic to humans whereas others cause very mild or no disease at all, indicates that there are viral factors that can determine the outcome of the infection. On the other hand, the fact that a certain hantavirus causes severe disease in humans and is cleared by the immune system, whereas infection of the natural host is non pathogenic and generally persistent, indicates that there are dramatic differences on the host level in coping with the infection. Therefore the pathogenicity for humans most probably depends on a combination of viral and host factors. From the virus' point of view the perfect situation is when the infected host lives a long life and sheds a lot of virus for a long time. In other words, killing or severely injuring the host is not a successful strategy, unless it is part of the viral transmission mechanism. Therefore the generally non-existent pathogenicity of hantaviruses for the natural hosts reflects the long-term co-evolution of the viruses with the different rodent species, in ways similarily to the co-evolution of humans and certain herpesviruses [204].

The lack of symptoms in the natural host as well as in many other animals has lead to difficulties in finding a proper animal model for studying hantavirus pathogenesis. A monkey model for the study of HFRS pathogenesis has been developed [205, 206] but these experiments are very expensive and demand advanced animal facilities. For studies of HCPS pathogenesis, a lethal model using Syrian hamsters has been developed [207]. Although hantaviruses are non-pathogenic in most natural hosts, one study has shown SNV infection to be pathogenic in deer mice [208], and another has found pathogenic markers in New York virus (NYV)-infected white-footed mice [209]. Whether these findings are unique for the two viruses and whether these findings can be benefited from in the study of HCPS or HFRS pathogenesis remains to be shown.

One major factor during the disease in infected humans is the increased capillary permeability that causes the vascular leakage and hemorrhagic manifestations. The mechanisms behind this dysfunction are, however, unclear. Hantaviruses are generally considered as non-cytopathic [106, 144, 210-218], although findings of high serum levels of lactate dehydrogenase (LDH) in patients indicate damaged tissue [219-221].

3.2 THE ROLE OF ENDOTHELIAL CELLS IN PATHOGENESIS

Endothelial cells are the epithelial cells lining the capillaries. They are the primary target for hantavirus infection and an increased permeability of the endothelial cell layer has been seen in lung tissues from lethal HCPS and HFRS cases [217]. However, since no endothelial cell damage has been observed due to hantavirus infections either *in vitro* or *in vivo*, the increased permeability most likely results from the influence of immune effector cells and/or cytokines.

An increase in the expression of several genes, especially in the early induction of IFN-stimulated genes and other genes which regulate immune cell responses, was observed after infection of endothelial cells with the pathogenic HTNV and NY-1 viruses, as compared to infection with the non-pathogenic PHV, which suggests a contribution to the pathogenesis [222].

Infection of human lung endothelial cells with HTNV or SNV induced an increased expression of the chemokines RANTES and IP-10, whereas no cytopathic effect (CPE) or increased permeability was observed [215]. Since these chemokines are chemotactic for mononuclear leucocytes this finding suggests that cellular immune responses rather than direct viral effects can explain the increased vascular permeability associated with HCPS and HFRS [215].

When endothelial cells where infected with either the non-pathogenic TULV or the pathogenic HTNV, a delayed production of the antiviral myxovirus resistance A (MxA) protein was seen after HTNV infection, which may contribute to the pathogenesis by allowing an early dissemination of the virus [223].

Further factors influencing pathogenicity include findings indicating that pathogenic hantaviruses use the αvβ3-integrin receptor for cell entry, whereas non-pathogenic hantaviruses instead bind to the αvβ1 integrin [88, 89, 224]. Pathogenic hantaviruses inhibit β3-integrin-directed endothelial migration, important for the maintainance of vascular permeability [225]. In addition, αvβ3-integrin regulates the function of a chemokine called vascular endothelial growth factor (VEGF), and a recent report shows that pathogenic hantaviruses sensitize endothelial cells to VEGF by inhibiting the function of αvβ3-integrin [226].

3.3 HUMAN IMMUNE RESPONSE AGAINST HANTAVIRUS INFECTION

Since there is considerable evidence that the pathogenicity of hantaviruses in humans to a large extent is immune-mediated [104, 218], a presentation of the immune response during hantavirus infection is here combined with its suggested contribution to the pathogenesis.

3.3.1 Innate immunity

Before an adaptive immune response is mounted, virus infection is counteracted by the innate immune responses. Viral RNA can function as pathogen-associated molecular patterns (PAMPs) that are recognized by pattern recognition receptors (PRRs) on various cells, such as epithelial and endothelial cells, and thereby activate the innate immune response [227]. The PAMP-PRR-interaction induces a production of antiviral cytokines called type I IFNs (IFN-α and IFN-β). IFN:s stimulate cells to produce antiviral proteins and antiviral molecules, like nitric oxide (NO) and peroxynitrate. Elevated levels of inducible nitric oxide synthase are found in the lungs of HCPS-patients [228]. Hantaviruses have been shown to be sensitive *in vitro* to IFNs [229], NO [230] as well as the IFN-inducible MxA protein [231, 232]. There are also indications that hantaviruses can interfere with the IFN-production [233] *in vitro*, and levels of IFN-λ are decreased in NE-patients [234].

Several proteins found in saliva constitute an important part of the innate immune response. These are further discussed in chapter 5 of this thesis.

Natural killer (NK) cells are important effector cells of the innate immune response, as they are able to kill infected cells by the induction of apoptosis. This is further discussed in chapter 4.

3.3.2 Cytokines

Cytokines are signal molecules of the immune system and can have several functions such as antiviral effects (e.g. IFN-α and IFN-β), to modulate or limit the immune response (e.g. IFN-γ and interleukin (IL)-10), to induce inflammation (e.g. TNF and IL-6) and to stimulate proliferation of T-cells (e.g. IL-2). Many cytokines also stimulate the production of other cytokines, which leads to a cascade of activation. Several findings indicate that cytokines play a major role in the immune response and possibly also in the pathogenesis in HFRS and HCPS. High levels of cytokine-producing cells have been found in lung tissues of patients with fatal HPS [235]. Elevated plasma levels of TNF, IL-6 and IL-10 were observed in NE patients as well as in PUUV infected cynomolgous macaques [205, 236] and TNF levels in NE patients were inversely correlated to blood pressure and symptoms [237]. Another finding that further supports TNF´s contribution to the pathogenesis is the finding that the TNF2 allele, which is associated with an increased TNF production during infection, is more common among NE patients as compared to the general population [237, 238]. Raftery and colleagues showed that HTNV could infect and activate dendritic cells, without signs of CPE or apoptosis, which resulted in an up-regulation of major histocompatibility complex (MHC) and adhesion molecules and induced a release of proinflammatory cytokines [239]. It was recently shown that there is a difference between male and female NE-patients in the induction of several cytokines [240].

3.3.3 Humoral response

Hantavirus infections induce strong humoral immune responses, with high titers of anti N and neutralizing antibodies, which are often already present at the onset of symptoms and therefore constitute good markers in diagnostics [241]. There is no evidence that hantavirus infections in humans can be persistent, yet PUUV-specific anti-N immunoglobulin G (IgG) and neutralizing anti-Gn and anti-Gc antibodies are detected several decades after infection [242]. High levels of PUUV-specific IgG_4 and IgA_1 antibodies, usually indicators of chronic infection, have been seen in late convalescent serum samples [243, 244]. However, apart from one study, where PUUV-RNA was detected from patients up to 16 days after onset of symptoms [245], all antigen detection or RT-PCR studies performed on samples drawn after the first week post onset of disease, have been negative [246-248].

High levels of virus specific IgM correlate to the appearance of clinical symptoms. As IgG responses may differ during the acute phase, it is recommended to rely on IgM for serodiagnosis of NE [249]. The decline in IgM levels during the convalescence phase usually coincides with a rise in IgG [250].

IgA is important for neutralizing virus in the mucosa during viral infections [251, 252] and elevated levels of total serum IgA have been found in acute-phase samples from NE-patients [244]. The importance of IgA during hantavirus infection is, however, not completely clear.

Compared to other viral diseases, levels of both total and virus specific IgE is increased in HFRS-patients infected with PUUV or DOBV [253-255]. The high levels of IgE found early after onset of disease have suggested a role for IgE in the pathogenesis of HFRS [253]. Further, plasma levels of PUUV-specific IgE correlated with the levels of soluble (s) CD23, a membrane glycoprotein, and a possible role for sCD23 in regulating the humoral immune response during HFRS has been suggested [256]. Interestingly, IgE can increase vascular permeability by triggering pro-inflammatory cytokines e.g. TNF and IL-1β [257] and by inducing vasoactive factors like serotonin and histamine [253]. However, levels of IgE do not correlate to the severity of the disease in NE-patients [104].

3.3.4 Adaptive cellular response

The adaptive cellular immune response consists of $CD4^+$ T-helper (Th) cells and $CD8^+$ cytotoxic T-lymphocytes (CTL). Although virus specific CTLs are important for the effective clearance of many virus infections, they have also been associated with apoptosis and severe tissue damage [258] and injury of endothelial cells [259]. CTL-induced cell death is discussed more thoroughly in chapter 4. Several studies reveal that the cellular immune response is of great importance during hantavirus infection in humans. Increased levels of activated T-cells have been observed in the circulation during HFRS [260]. The levels of CTL-responses are correlated with the severity of SNV infections [261]. Infiltrations of mononuclear cells containing CTLs have been seen in acute HFRS [262, 263] and HCPS [217, 235] patients. CTLs are capable of producing cytokines, such as TNF and IFN-γ, which can stimulate inflammation and induce apoptosis.

T-cells recognize viral peptides that are presented on human leucocyte antigen (HLA)-molecules on the surface of infected cells or antigen presenting cells. HLA molecules occur in various haplotypes that differ in their ability to present different peptides. One finding which may have implications for many parts of the immune system during a hantavirus infection is that the occurrence of certain HLA-haplotypes correlate with the severity of disease in NE patients [264-266]. HLA-haplotypes have also been correlated with an increased CTL response in SNV-infected patients with severe HCPS [235].

3.3.5 Immune complexes and complement activation

Immune complexes formed by immunoglobulins and antigens can activate the complement system and have been suggested to be the cause of pathogenicity in various autoimmune [267] and infectious diseases such as hepatitis [268] and mononucleosis [269]. Immune complexes have been found in biopsies and autopsy tissues from HFRS patients [270, 271] and in the plasma of HCPS patients [272]. However, whether the occurrence of immune complexes correlates with the severity of HFRS and HCPS is still not clear [273, 274].

4 APOPTOSIS

Principally, cell death can be divided into two main entities, necrosis and apoptosis, which differ fundamentally in several aspects. Necrotic cell death is inflammatory, energy independent, less regulated and results in cell swelling, membrane rupture and leakage of lysosomal enzymes. Apoptosis on the other hand, is non-inflammatory, energy dependent, highly regulated by the activation of specific proteases and leads ultimately to cell shrinkage and the formation of apoptotic bodies, a process during which membrane integrity is continuously maintained.

4.1 HISTORY

Knowledge about programmed cell death first arose in the field of developmental biology and the first insight into the mechanisms that regulate apoptosis came from Sulston and Horvitz [275] while studying the development of the nematode *Caenorhabditis elegans*. However, not until Vaux and colleagues discovered that the human bcl-2 gene was able to block apoptosis in *C. elegans* [276] was it realized how conserved the mechanisms involved in apoptosis were among both invertebrates and humans, and what possible applications the discoveries made in *C. elegans* had in human medicine [277].

4.2 APOPTOSIS PATHWAYS

Independent of the immune system, apoptosis can be induced by different stimuli, such as growth factor withdrawal, DNA damage, starvation, drugs, UV-irradiation or viral infection. This type of apoptosis is usually initiated by actions associated to the mitochondria [277].

Alternatively, apoptosis can be induced by CTL and NK cells as an important mechanism by which the immune system eliminates virus infected or transformed cells [278]. Immune-mediated apoptosis is divided into two main pathways: the receptor-mediated and the granule-mediated (Fig. 6).

The receptor-mediated pathway involves the binding of different cell-death inducing ligands to their corresponding receptor on the target cell. Three major receptor/ligand pairs have been identified: 1) Fas and Fas ligand (FasL) [279], 2) TNF-related apoptosis inducing ligand receptor (TRAIL-R) and TRAIL [280] and 3) TNF receptor (TNF-R) and TNF [281]. Ligand binding induces the association of the receptor with the adaptor proteins FADD or TRADD [282, 283] and procaspase 8, an inactive precursor of a proteolytic enzyme. This formation, which is called the death inducing signaling complex (DISC), activates caspase 8, which cleaves the effector caspase 3, which in turn induces a cascade of effector caspase activation [284]. These caspases degrade several key cellular substrates, and this ultimately leads to nuclear fragmentation and cell death.

In granule-mediated cell death, proteolytic enzymes, such as granzyme B (GrB), are delivered to the target cell via an immunological synapse [285]. The entry of GrB is mediated by the pore forming protein perforin through yet unknown mechanisms.

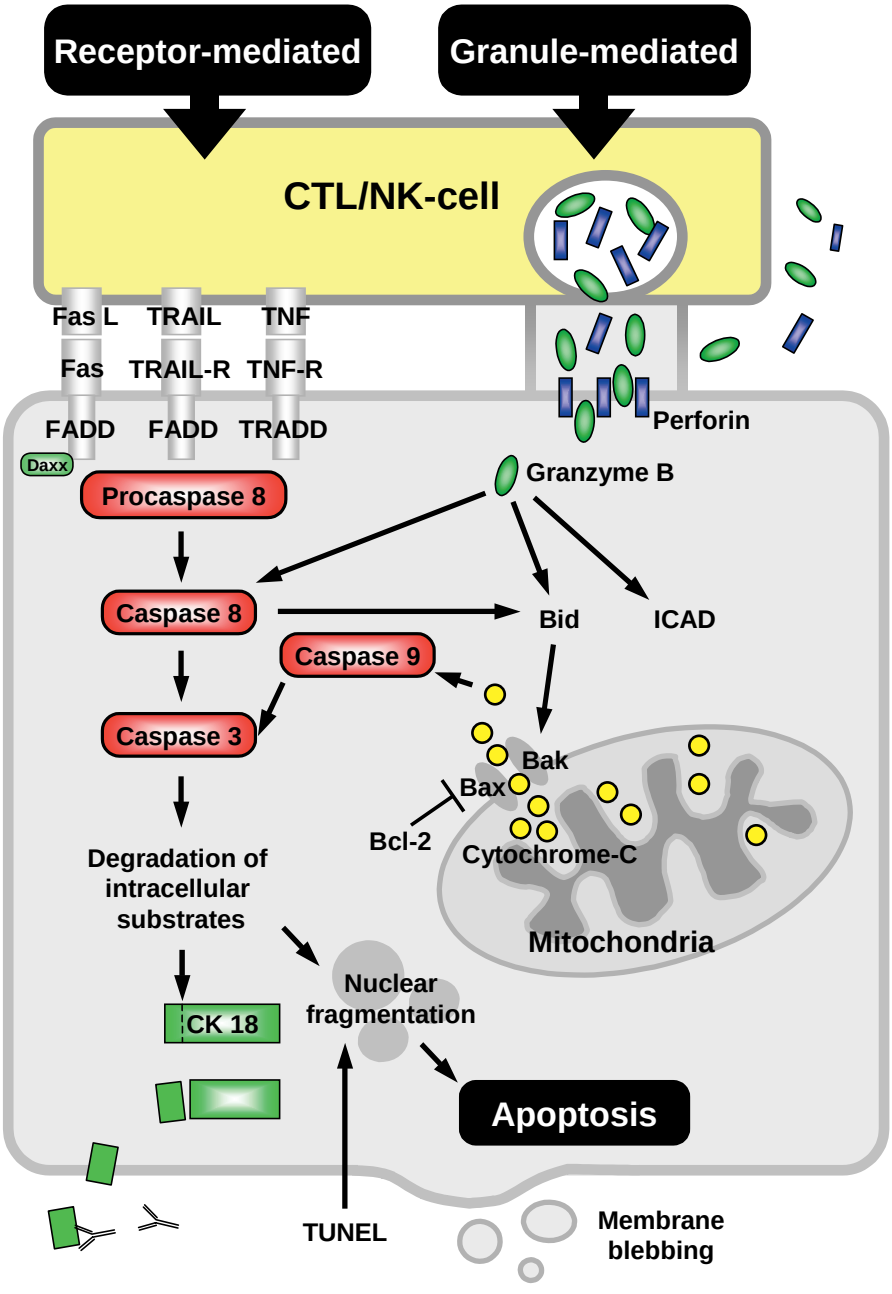

Figure 6. Pathways of immune-mediated apoptosis.

During this process, some of the GrB and perforin most probably leak out into the circulation and can be detected in patient serum, as has been observed in patients with an ongoing CTL-response due to e.g. Epstain-Barr (EBV) or HIV infection [286, 287]. Once inside the target cell, GrB promotes cell death either via direct cleavage of caspases, such as caspase 3 and 8, or by promoting mitochondrial permeabilization [278]. Mitochondrial-mediated apoptosis is regulated by the Bcl-2 family of proteins. GrB can cleave one proapoptotic member of this family, Bid, which thereby starts the oligomerization of two other Bcl-2 family members, Bak and Bax. These proteins form a pore in the outer mitochondrial membrane through which cytochrome-c can be released into the cytosol [288]. This release is inhibited by the anti-apoptotic Bcl-2. Once in the cytosol, cytochrome c activates caspase 9, which in turn cleaves caspase 3. The receptor-mediated induction of apoptosis can also involve the mitochondria via the caspase 8 induced activation of Bid [289].

GrB can also induce apoptosis in the absence of caspases via the cleavage of other substrates such as the inhibitor of caspase activated deoxyribonuclease (ICAD), which leads to internucleosomal degradation of DNA [290].

The intermediate filament protein cytokeratin 18 (CK 18), expressed in simple epithelial cells, such as endothelium, is one of the proteins that are cleaved by caspase 8. During apoptosis, the cleaved form of CK 18 leaks out from the dying cell and can be detected by the use of specific antibodies [291, 292]. This method has been used to monitor apoptosis in cancer patient sera [293]. The free 3´-ends that are produced during apoptosis-induced DNA fragmentation can be detected by the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) assay, in which modified nucleotides are added in an enzymatic reaction [294].

4.3 VIRUSES AND APOPTOSIS

Apoptosis as a consequence of viral infection can be either host-induced as a means of controlling the infection, or virus-induced in order to promote virus progeny. The simple fact that apoptosis is detected during a viral infection tells nothing about which of these two mechanisms that lies behind it. There are also numerous examples of viruses that have evolved mechanisms to manipulate the host's cell death program by blocking apoptosis [295]. Examples are adenovirus and EBV expression of viral proteins that mimic the anti-apoptotic host protein Bcl-2 [296, 297]. The importance of these mechanisms have been shown by the production of viral constructs deleted for such genes, which have generated attenuated viruses [298, 299].

Although apoptosis can be detrimental for the virus during replication, it can be beneficial when induced after virus assembly. Apoptosis induced at the culmination of a lytic infection can constitute a mechanism by which viruses spread to other cells without causing inflammation. Viruses can be transferred to phagocytotic cells carried in apoptotic bodies and thereby avoid recognition by neutralizing antibodies [298]. Using EM, it has been shown that adenovirus have evolved such a strategy for viral dissemination [300]. Structural proteins can act as super ligands for death receptors as exemplified by HIV gp120, which alone is able to induce apoptosis in non-infected bystander cells. It thereby contributes to the depletion of lymphoid and neuronal cells,

whereas infected cells are resistant to apoptosis since they express another viral protein, tat, which counteracts apoptosis [301].

4.3.1 Hantaviruses and apoptosis

Apoptosis and its possible contribution to hantavirus pathogenesis has been investigated during the last decade. One can speculate why apoptosis would occur as a consequence of hantavirus infection in humans and consider the following three reasons:

1) Hantavirus induces apoptosis "against the cell's will" because the virus benefits from it for some reason. Since humans are generally a dead end host, the evolution of such a strategy would probably have to have taken place in the rodent host.
2) Hantavirus activity in the cell gives rise to a cell autonomous apoptosis, as a host defense mechanism, independent of the immune system. Apoptosis due to this reason would probably not be induced in non-infected bystander cells, unless the infected cell produces apoptosis-inducing signaling molecules.
3) The immune system senses the hantavirus infection and apoptosis is induced by CTL and/or NK cells as a way to clear the infection. Although this action is host induced, an over-reaction could lead to an immune-mediated pathogenesis.

Kang and colleagues were the first to report hantavirus induced apoptosis. They found that both HTNV and the non-pathogenic PHV induced apoptosis in Vero E6 cells [302]. The HTNV-infected cells also showed down-regulated levels of the anti apoptotic Bcl-2 protein. TULV has been reported to induce apoptosis *in vitro* involving an interaction with the Fas-mediated apoptosis enhancer Daxx [303]. However, whether Daxx is associated to pro- or anti- apoptotic mechanisms has been questioned elsewhere [304]. Further, TULV-infection induced an activation of TNF and caspase-8 [305] and ER-stress [306]. Markotic and colleagues reported detection of both CPE and apoptosis in HEK293-cells after infection with ANDV, SEOV and HTNV [307]. In those experiments apoptosis was however mainly seen in bystander cells and was not connected to an increased expression on the mRNA-level of FasL or TRAIL. Additionally, HEK293 has been reported to be CPE-inclined [308].

There have also been reports where hantaviruses induced no or very low levels of apoptosis *in vitro*. SNV and PHV have been reported not to cause apoptosis after infection of human umbilical vein endothelial cells (HUVECs) [309] and no apoptosis was seen after PUUV, SAAV, DOBV or HTNV infection of confluent Vero E6 cells [paper III].

It is hard to draw comprehensive conclusions from these to some extent contradictory studies. Apoptotic events may also be depending on cell health and passage status, and *in vitro* studies on apoptosis have their obvious limitations in explaining hantavirus pathogenesis in humans. However, during recent years increasing evidence has been found for the occurrence of apoptosis in HFRS patients. An increase in Fas/FasL and an activation of both initial- and effector- caspases were observed in PBMC drawn from acute and convalescent HFRS patients [310]. Positive cleaved poly (ADP-ribose)

polymerase (PARP) immunohistochemistry staining, a sign of apoptosis, was observed in kidney biopsies from 4 out of 5 NE patients [311]. Elevated levels of the soluble and membrane bound forms of the apoptosis inducing ligands FasL, TRAIL and TNF, were detected in HTNV infected HFRS patients [312]. Serum levels of the epithelial cell apoptosis marker caspase-cleaved CK 18 were elevated during the acute phase in 14 of 18 NE-patients [paper IV].

It is too early to clearly define the role for apoptosis in the pathogenesis of HFRS and HCPS but one can suggest that the apoptosis seen in HFRS-patients contributes to the cellular damage that causes the increased capillary permeability, which in turn leads to the hemorrhagic symptoms. It can, however not be excluded that the absence of apoptosis seen in some studies is due to a possible ability of hantaviruses to block apoptosis, although there has so far been no reports on such a mechanism.

5 PERSON-TO-PERSON TRANSMISSION

Zoonotic diseases are generally transmitted from animals to humans and only rarely between humans. Humans have long been considered dead end hosts for hantaviruses, but with the increasing evidence for person-to-person transmission of ANDV [313-317], this dogma is now about to be rewritten.

5.1 ANDES HANTAVIRUS

ANDV is the only known hantavirus where person-to-person transmission has been documented. The first reports on suspected interpersonal transmission came from Argentina and Chile 1996-98 and were based on epidemiological data where clusters of cases were studied to find the probable sources of infection [313, 316, 317]. The time interval for onset of disease between patients in a cluster can provide a clue to possible person-to-person transmission (Fig. 7). Interpersonal transmission should be suspected when approximately three weeks pass between onset of symptoms for an index case and epidemiologically related persons, whereas a less than two week interval suggests a common rodent source [314]. As it is often difficult to completely rule out the possibility that patients in a cluster have been exposed to the same rodent source, it was an interesting finding when in 1998, Padula and colleagues found the first molecular evidence for person-to-person transmission [315]. In this study, a chain of 16 epidemiologically related patients appeared to be infected with ANDV carrying an identical nucleotide sequence (a total of 1075 nucleotides from the S and M segments). One hypothetical explanation to this fact could be that the patients were infected with a genetically identical hantavirus, either from the same rodent or from rodents of the same local population. However, given the large geographical distance between the residences of the patients, sometimes up to 1400 km, the most reasonable explanation is that they were infected through person-to-person transmission while hospitalized [315].

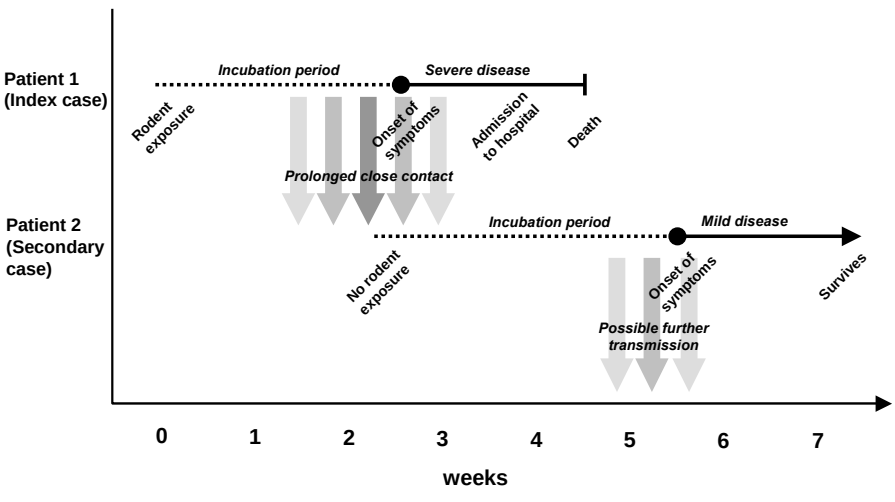

Fig. 7. Suggested timescale for person-to-person transmission of ANDV.

Interpersonal transmission of ANDV appears to be limited to a short period in the late prodrome or the early cardiopulmonary phase and probably demands close contact with an infected person [314, 318]. At the time of the patients' admission to the hospital, the disease has most often progressed to the cardiopulmonary phase, which together with the protective equipment in use, can explain the few cases among medical personnel [314, 318]. Apart from the 1996 outbreak in Argentina, where one receptionist and five doctors were infected, ANDV infections in hospital workers are rare events and the seroprevalence among healthcare workers is not different from that seen in the general population [319].

There are facts indicating that severe, in contrast to mild, cases of HCPS are more likely to generate secondary cases and that the secondary cases in turn often have a milder course with fewer deaths as compared to the index cases [314]. Whether this is due to differences in the viral load from rodents and humans and/or due to changes in the virus as a result of host change, remains to be shown.

5.1.1 How is the virus transmitted between humans?

Oral transmission is believed to be the main factor for ANDV transmission among pygmy rice rats [148] and studies on primary airway epithelial cells from hamster have suggested an important role for the infection of respiratory epithelium in the early or prodrome phase of disease in humans as well as for transmission [214]. Viral shedding from ANDV patients has not been studied, but ANDV RNA has been detected in the salivary glands of ANDV patients in Argentina [320]. Together with the finding of virus in intraalveolar pulmonary macrophages [316] this suggests that ANDV can be shed via saliva or during coughing. However, the limited number of recorded cases of person-to-person transmission suggests that a close and long contact is needed for virus to be transmitted. The study of case clusters can give information of transmission rates and risk factors. In an epidemiological prospective study during a HCPS outbreak in Chile during 1997-1998, 76 index cases were followed [321]. When 421 household contacts to these patients where observed, 16 new cases were found, 6 of which showed presence of ANDV RNA in their blood up to 2 weeks before onset of symptoms or presence of antibodies. Among these 16 cases, 3 were definite, 9 were probable and 2 were possible person-to-person transmissions. Among these household contacts, sex partners to the index cases had a 17.6% risk of developing HCPS compared to 1.2% among non-sex partners [321].

5.2 OTHER HANTAVIRUSES?

There is so far no evidence that hantaviruses, other than ANDV, have been transmitted from person-to-person, although this might of course be due to the lack of investigations. Also for SNV the number of cases among healthcare personnel seems to be limited [322]. If the suspected early and short period of contagiousness for ANDV also is true for SNV, this does not necessarily indicate that interpersonal transmission is unlikely or impossible. However, since interpersonal transmission of ANDV became evident, SNV and different clusters of HCPS in the USA, have been studied quite extensively and it has so far not been possible to exclude a common contact with a rodent reservoir [323]. Further, for HTNV there were no reported cases of infections among healthcare personnel during the Korean War [324]. So far no clusters of NE

cases, where direct exposure to rodent excreta can be excluded, have occurred in Sweden [paper V].

When studying epidemiological data to find support for person-to-person transmission of hantavirus it is important to consider the extensive *ex vivo* stability of the virions that has been shown experimentally [191, paper II]. Although a household contact to an index case, perhaps living far from the area with endemic rodents, states no contact with rodents or rodent droppings, it might still be theoretically possible that virus in dried aerosols has been transported on the patients clothing and thereby been exposed to the other members of the family, perhaps after several days of incubation. Molecular evidence would not help to find the true pattern of transmission in this case. Further, the generated time interval between the onsets of disease for the index patient and the family member (due to the virus incubation and the possibly lower dose of inhaled virus) would falsely point towards an interpersonal transmission.

5.3 ANTI-VIRAL EFFECT OF HUMAN SALIVA

The most likely route for a person-to-person transmission of hantaviruses is via the saliva. Human saliva has many important functions such as mechanical cleansing of the oral cavity, buffer capacity, taste, digestion of starch and antimicrobial mechanisms [325]. Apart from water (99%), saliva consists of many substances, such as antibodies (mainly IgA) and various non-specific proteins with a documented effect against microorganisms (Table 3). A healthy person produces about 1 to 1.5 liters of saliva each day [326].

Table 3. A selection of salivary components with documented antiviral activity

Component	Antiviral mechanism	Reference
Antibodies, mainly IgA	Neutralize and inactivate viruses	[244, 327]
Lactoferrin	Binds iron, binds host cell and viral particles, delays viral protein synthesis, inhibits viral entry and shedding.	[328-330]
Lysosyme	Destroys viral membranes.	[331, 332]
Secretory leucocyte protease inhibitor (SLPI)	Limits viral entry.	[333, 334]
Mucin	Aggregates viral particles.	[335]

Despite the antiviral mechanisms of human saliva there are several examples of viruses that are normally transmitted via saliva e.g. herpes simplex virus and EBV [336, 337]. There are also examples of viruses that are detected in saliva but where evidence of oral transmission is rare or absent. Hepatitis C virus (HCV) RNA has been detected in the saliva from 50% of infected patients [338] and the infectivity of the saliva has been proven by the detection of HCV RNA in the serum of a chimpanzee after inoculation with HCV-infected human saliva [339]. However, although there is evidence that non-

sexual intrafamiliar transmission of HCV has occurred [340], probably via other biological fluids than blood [341], this kind of transmission has been reported to be extremely rare or absent [342, 343] and it has been suggested that saliva can attenuate or abolish the infective capacity of HCV and that the HCV RNA found in saliva originates from non-infectious fragments of the virus [344].

HIV and possible transmission via saliva has also been studied extensively. HIV is detected in saliva from a proportion of HIV patients [345, 346], but exposure to HIV-infected saliva poses a far lower risk, compared to blood exposure as extremely few cases of oral transmission are reported from epidemiological studies [327]. One reason to this might be that HIV in saliva is inhibited by several antiviral substances, of which the secretory leucocyte protease inhibitor (SLPI) seems to be of great importance [327].

6 AIMS

The general aims of this work have been to gain better understanding of hantavirus transmission and to better understand the pathogenesis in infected humans. The specific aims have been:

- To investigate the patterns of Puumala virus shedding from the natural host.
- To evaluate the *ex vivo* stability of Hantaan virus in comparison to arthropod-borne members of the *Bunyaviridae* family.
- To investigate if apoptosis is induced by hantavirus infection *in vitro* and in patients.
- To evaluate the possibility of person-to-person transmission of Puumala virus.
- To study the effect of human saliva against hantavirus.

7 RESULTS AND DISCUSSION

7.1 THE SHEDDING OF PUUMALA VIRUS FROM THE NATURAL HOST (PAPER I)

Virus shedding from the host is of major importance for the transmission efficacy of hantaviruses. It has long been known that PUUV is shed in bank vole saliva, urine and feces [175]. However, how the levels of shed virus change over time in an infected bank vole has not been studied in detail. In this study, we subcutaneously inoculated 10 bank voles with PUUV and sampled excretions regularly until day 133 post infection (PI). Levels of shed viral RNA peaked within 11-28, 14-21 and 11-28 days post infection for saliva, urine and feces, respectively. The latest detection of viral RNA was 84, 44 and 44 days post infection, in saliva, urine and feces respectively. In contrast, viral RNA was detected in blood from five out of six animals at day 133 post infection, suggesting that bank voles, although persistently infected, secrete virus only during a limited time of the infection. As detection of RNA does not necessarily correspond to the occurrence of infectious virions, we wanted to test if the RNA-positive excretion samples also contained infectious virus. We also wanted to test if intranasal inoculation was a possible infection route for all types of excretions. Therefore, a subset of the PUUV RNA positive urine, feces and saliva samples, collected from the subcutaneously inoculated bank voles, were administered intranasally to 14 naïve bank voles. In total, 7 (2/4, 2/5 and 3/5 in the groups given saliva, urine or feces, respectively) out of these 14 bank voles seroconverted, indicating that all these three transmission routes may occur in nature and that also rodent saliva might play a role in transmission to humans.

The 14 intranasally inoculated animals were also sampled for saliva, urine and feces and by running real-time RT-PCR on a selection of these samples, we showed that all types of excretions enabled subsequent detection of viral RNA, which indicates that virions excreted via different routes do not show restricted tropism for particular tissues.

When serum drawn at day 42 PI from the intranasally inoculated bank voles was analyzed by ELISA, we observed that seven out of 14 bank voles had seroconverted. Interestingly, only one of the animals was positive according to a previous ELISA performed on serum from day 21 PI. The reason for this late seroconversion in the majority of the bank voles might be that the bank vole saliva, urine and fecal samples used for intranasal inoculation contained relatively low virus doses.

7.2 *EX VIVO* STABILITY OF HANTAAN VIRUS (PAPER II)

For viruses that are transmitted via the environment, a prolonged *ex vivo* stability could significantly increase the transmission efficacy, especially when the density of susceptible hosts is low [188]. The *Bunyaviridae* family consists of viruses that are either arthropod-borne, which means that they are never exposed to an *ex vivo* environment, or rodent-borne, and thus generally transmitted via the environment. It is reasonable to suspect that the rodent-borne viruses would derive an advantage from

having a longer *ex vivo* stability, as excreted virus would infect more new hosts before infectivity is lost. To test this hypothesis we compared the stability of HTNV (a rodent-borne hantavirus), Sandfly fever Sicilian virus (SFSV, a sandfly-borne phlebovirus) and Crimean Congo hemorrhagic fever virus (CCHFV, a tick-borne nairovirus). These viruses are morphologically similar, with a spherical structure and a lipid envelope. We studied if these viruses differed regarding the *ex vivo* stability when exposed to different temperatures (4, 20 and 37ºC) and drying at 20ºC. We observed a clearly different stability in wet conditions, particularly at 4ºC, where infectious SFSV, HTNV and CCHFV were detectable after 528, 96 and 15 days, respectively. All three viruses were equally sensitive to drying, as shown by drying on aluminium discs. Infectious particles, representing 6-14% of the input concentration, were extracted from the three assayed viruses after 90 minutes. We were not able to detect any infectious virus from any of the genera from discs incubated for 24 hours or longer.

To investigate possible differences in the membrane integrity, we also tested the susceptibility of the viruses to inactivation by different concentrations of ethanol. All viruses where completely inactivated after two minutes in 40% ethanol whereas HTNV and SFSV partially survived two minutes in 30% ethanol. A lower concentration (10%) of ethanol had no detectable effect on the inactivation of any of the viruses. These observations might have implications for laboratory work where decontamination with ethanol is a commonly used method to inactivate virus.

In order to find what caused the decreased infectivity over time, we performed EM studies on HTNV, SFSV and CCHFV stored at 37ºC until infectivity was lost. We found approximately the same virus concentrations in non-incubated (infectious) and incubated (non infectious) samples. However, the abnormal shape and density of the virions in the incubated samples indicates that inactivation was due to disruption of the virus particles. The EM studies also revealed the presence of SFSV aggregates consisting of 20-300 virions. The concentration of these aggregates corresponded well to the concentration of infectious units observed in our samples. This indicates that we, in the plaque assay, in fact were measuring the infection by these aggregates and not by individual virions. It was not possible for us to increase the virus titer by vortexing, but if an adequate method is found, the titers may increase approximately a hundred- fold, provided that all the virus particles within the aggregates are infectious. Whether this aggregation phenomenon is beneficial in terms of increased stability or immune escape of the virus remains to be shown.

The examined viruses within the *Bunyaviridae* family showed great variation in *ex vivo* stability at wet conditions, while they were equally sensitive to drying. The results indicate that HTNV, although being non vector-borne, does not seem to have evolved a higher ability to survive *ex vivo* as compared to CCHFV or SFSV.

7.3 ABSENCE OF APOPTOSIS *IN VITRO* (PAPER III)

Hantaviruses are known to cause little or no CPE *in vitro*, but as HTNV and PHV were reported to cause apoptosis in Vero E6 cells in 1999 [302] we wanted to test additional hantaviruses and study if there was a difference between more pathogenic and less pathogenic hantaviruses in the ability to induce apoptosis. Therefore, we infected

confluent Vero E6 cells with HTNV, DOBV, SAAV and PUUV. However, when studying signs of cell death and apoptosis at three, six, nine or twelve days after infection, we found no differences in the percentage of adherent cells, or cells with condensed nuclei between the different hantaviruses, and surprisingly also not when comparing the virus-infected to the non-infected cells. Furthermore, no differences in the percentage of cells with inter-nucleosomal cleavage of DNA, between uninfected and HTNV-infected cells, could be detected using the TUNEL assay. Possibly, slightly more apoptotic cells, but never more than 5%, were detected after HTNV infection of non-confluent cells as compared to the negative control. Further, we could verify the earlier reported results of TULV-induced apoptosis [305], suggesting that non-pathogenic hantaviruses might differ from HFRS-causing strains regarding induction of apoptosis.

The explanation to the differences between our results and those presented by Kang et al. [302] is far from obvious, as the experimental procedures seem quite similar. One explanation could be that although the same virus strain was used, mutations might have occurred during propagation, resulting in differences in induction of apoptosis. Alternatively, the differences are depending on the cells, as the ability to induce apoptosis in a cell culture has been reported to be dependent on the passage history of the infected cells [307].

In conclusion, the finding that HFRS-causing hantaviruses in our experiments are very poor inducers of apoptosis *in vitro* might indicate that if the mechanisms behind the increased capillary permeability involved in hantavirus pathogenicity is due apoptosis, the apoptosis is likely to be induced by immune cells rather than stimulated by hantavirus infection alone.

7.4 INDUCTION OF APOPTOSIS *IN VIVO* (PAPER IV)

Although pathogenic hantaviruses are not cytotoxic, increased levels of serum LDH, aspartate aminotransferase, and alanine aminotransferase, indicative of cellular damage, are observed in patients [219-221]. This shows that the cellular membrane integrity is disturbed during infection. The pathogenesis is believed to be immune-mediated and special attention has been drawn to the cellular response [218]. Therefore we wanted to investigate if apoptosis could be observed in HFRS-patients during the acute phase of disease.

This study was based on two phenomena that occur during CTL induced target cell apoptosis. 1) When apoptosis is induced via the granule exocytosis pathway, some of the perforin and granzymes probably find their way out of the synapse and into the circulation [347]. 2) During epithelial cell apoptosis, CK 18 is cleaved by caspases and diffuses into serum where it can be detected by a specific antibody that only recognizes the caspase-cleaved form of CK18 [291].

We studied acute and convalescent serum samples from 18 patients hospitalized with a laboratory verified PUUV-infection. The levels of caspase-cleaved CK18, perforin, and GrB were analyzed by ELISA.

All patients showed increased levels of serum LDH during the acute phase of infection and there were significantly higher levels of caspase-cleaved CK 18, extracellular perforin and GrB in the acute, as compared to the convalescent, sera. This indicates that apoptosis is induced in cells of the epithelial cell lineage during the acute phase of HFRS. The level of LDH correlated significantly with that of epithelial cell apoptosis, which suggests that the majority of the cell damage observed during hantavirus infection, is caused by apoptosis.

The levels of LDH and perforin correlated significantly, suggesting that hantavirus specific CTLs and/or NK cells might be involved in causing the observed cell damage during HFRS/HCPS. Interestingly, the levels of GrB and LDH did not correlate. This is in line with the proposed functions of perforin and GrB during the killing of target cells by cytotoxic cells: although GrB induces the apoptosis, perforin is needed for GrB to enter the cell [285].

The levels of caspase-cleaved CK 18 did not correlate significantly to the levels of perforin or GrB, which might be due to a delay between induction of apoptosis and release of granular enzymes. Although it could be speculated that the increased vascular permeability observed during HFRS/HCPS is due to apoptosis caused by hantavirus-specific CTLs, this remains to be clearly shown. Apoptosis of epithelial cells other than endothelial cells, might also contribute to the increased levels of caspase-cleaved CK18.

In conclusion, the capillary leakage during HFRS/HPS could be due to apoptosis, and the strong hantavirus-specific CTL responses observed [248, 260, 348] may contribute significantly to the damage.

7.5 PUUMALA VIRUS RNA IN HUMAN SALIVA (PAPERS V AND VI)

ANDV is so far the only hantavirus with evidence for a person-to-person transmission [315] and deep kissing with the index case has been suggested as a possible risk-factor for infection [321]. This, together with the finding of relatively high levels of PUUV RNA in saliva collected from bank voles [paper I] made us investigate whether PUUV RNA could be detected in saliva from NE patients. Therefore, we collected saliva and plasma from 14 hospitalized NE patients with verified PUUV infection. Using real-time RT-PCR, PUUV RNA was detected in saliva from 10 of these patients with samples taken between 2-9 days post onset of symptoms. All patients were positive for PUUV RNA in plasma and the PUUV S-segment sequences from saliva and plasma of the same patients were identical.

The next step was to investigate if the detected viral RNA corresponded to infectious virus. Using Vero E6 cells, we were not able to isolate any virus from the saliva samples. Furthermore, we used saliva from all 14 patients to inoculate bank voles. Forty-two days after inoculation bank voles were bled, and serum analyzed for PUUV-specific IgG-antibodies. Seroconversion was however not observed in any of the inoculated bank voles, suggesting that the saliva did not contain infectious particles.

7.6 ANTI HANTAVIRUS EFFECT OF HUMAN SALIVA (PAPER VI)

To test if human saliva interferes with hantavirus replication, we studied the effect of saliva and salivary proteins on hantavirus replication. Whole saliva collected from healthy individuals partly reduced HTNV infectivity when incubated for 1h at 37ºC, whereas pre-incubation of cells with saliva before infection had no effect on HTNV infectivity, suggesting that the observed antiviral effect of saliva was caused by a direct effect of salivary components on the virus particles. Furthermore, HTNV was resistant against the antiviral capacity of several salivary proteins (at concentrations above those normally observed in saliva) like histatin 5, lysozyme, lactoferrin, and SLPI, but was partly inhibited by mucin. The observed inhibition of HTNV by mucin was concentration-dependent.

In conclusion, we found no evidence of infectious virus in patient saliva, which might be at least partly due to the anti-HTNV activity of human saliva. However, the *in vitro* experiments showed that HTNV was insensitive to several antiviral salivary proteins, and was not completely inactivated by human saliva. Therefore, it remains to be shown if human saliva might contain infectious hantavirus particles early during infection, for instance before neutralizing antibodies are produced.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA (SUMMARY IN SWEDISH)

Vad är ett virus?
Virus är inte levande till skillnad från exempelvis bakterier. Virus är endast små protein/fett-strukturer som i sin tur omsluter virusets gener. Virus är därför helt beroende av levande celler för att produceras och spridas. Virusets gener innehåller information om hur den infekterade cellen ska bygga ihop nya virus. Det finns många olika sorters virus som kan infektera människor, djur eller till och med bakterier.

Vad är hantavirus?
Den grupp av virus som kallas hantavirus är ämnet för denna avhandling. Hantavirus sprids av gnagare, framförallt sorkar, möss och råttor, runt om i världen. Viruset utsöndras via gnagarens saliv, urin och avföring och smittar på så sätt andra gnagare eller oss människor om vi råkar andas in damm som förorenats av t.ex. sorkar. De naturliga värddjuren, d.v.s. gnagarna, blir inte sjuka av viruset, men det kan vi människor bli. Om vi blir sjuka och vilka symptom vi får beror till stor del på vilken sorts hantavirus vi smittats av. Det finns nämligen en mängd olika kända varianter, och de flesta sprids av en egen gnagarart. De hantavirus som finns i Nord- och Sydamerika kan ge allvarliga lungsjukdomar medan hantavirus i Europa och Asien snarare ger allvarliga njursymptom. En mildare sjukdomsvariant kallas sorkfeber och orsakas av ett hantavirus, Puumala-virus, som sprids av skogssorkar, mestadels i norra Sverige och övriga delar av norra Europa. Gemensamt för de olika sjukdomstyperna är att blodkärlen börjar läcka och att blödningar lätt uppstår. Därför kallas hantavirus ibland för blödarfebervirus. De exakta bakomliggande orsakerna till dessa blödningar är dock inte kända. Det finns i dagsläget varken ett effektivt vaccin mot hantavirus eller någon specifik behandlingsmetod för sjukdomarna som det orsakar.

Vad handlar avhandlingen om?
I denna avhandling har vi arbetat både med Puumalavirus och med andra hantavirus när vi sökt svar på följande frågor:

Hur utsöndrar sorkar viruset?
Kunskap om hur virus utsöndras från värddjuren kan hjälpa oss att förstå hur virus sprids i naturen och därigenom kan vi få möjlighet att förutsäga förekomsten av hantavirus-infektion hos värddjuren. Därmed kan vi också bättre förutspå risken för att människor ska smittas. Genom att mäta mängden virusgener har vi undersökt hur Puumalavirus utsöndras från skogssorkar i fångenskap. Vi upptäckte att utsöndringen var som störst ungefär tre veckor efter att djuren infekterats. Det verkade också som att saliv innehåller förhållandevis mycket virus. Vi visade vidare att såväl saliv, urin som avföring kunde infektera nya sorkar när de smittades via inandning genom nosen, dvs. så som man tror att smittspridning ofta sker i naturen. Detta tyder på att vi människor kan smittas av sorkarnas urin och avföring, men även via deras saliv, exempelvis när vi äter mat som sorkar smakat på.

Hur länge är hantavirus smittsamt i miljön?
Eftersom hantavirus sprids via miljön gynnas de av att vara stabila. Ju längre ett virus är intakt och därmed smittsamt, desto större sannolikhet är det att en passerande oinfekterad gnagare smittas. Virus som smittar fler individer kommer att öka i antal och därmed spridas mer effektivt. Hantavirus har besläktade virus som i stället för att spridas via miljön överförs av insekter från värddjur till värddjur. Vi ville undersöka om hantavirus har utvecklat en bättre stabilitet än dessa insektsburna virus som ju egentligen aldrig förekommer fritt i miljön. När virus utsattes för intorkning så försvann infektiviteten helt inom ett dygn. I fuktig miljö kunde hantavirus däremot fortfarande infektera celler efter nästan 100 dagar. Det verkade dock inte som om hantavirus var mer stabilt än de insektsburna virussläktingarna. Resultaten från denna studie kan också användas som grund för ett säkrare laborativt arbete med dessa virus.

Varför blir vi människor sjuka av hantavirus när inte gnagarna blir det?
En huvudsaklig anledning till detta är antagligen att virusets långa utveckling tillsammans med ”sin” gnagare har bidragit till en ömsesidig anpassning. Det är egentligen inte lönsamt för viruset att orsaka sjukdom, utan det viktiga är att spridas, d.v.s. att föra det genetiska materialet vidare. Vi människor däremot, smittas mer av tillfällighet och viruset är helt oanpassat till oss, vilket verkar få en del allvarliga konsekvenser. Vad sker då i kroppen? Många virus orsakar sjukdom genom att de dödar kroppens celler. När man odlar hantavirus i cellkultur sker dock ingen markant celldöd. Därför har man traditionellt inte trott att det är därför man blir sjuk av hantavirus. Men celler kan dö på olika sätt. Så kallad apoptos, en mycket kontrollerad celldöd, är inte lika märkbar. Apoptos kan vara ett sätt för kroppen att försvara sig mot en virusinfektion. Celler i kroppens immunförsvar kan via apoptos döda de virusinfekterade cellerna och därigenom stoppa virusproduktionen. Vi har undersökt om hantavirus leder till apoptos. När vi infekterade celler i provrör kunde vi inte se någon ökning av apoptos. Men när vi i stället undersökte blodprover från sorkfeberpatienter upptäckte vi att apoptos förekom under sjukdomens akuta skede. Kanske är det så att kroppens immunförsvar försöker döda de hantavirusinfekterade cellerna med hjälp av apoptos, men att reaktionen blir för kraftig och rent av skadlig för människan?

Smittar hantavirus mellan människor?
Man har länge kallat människan för en återvändsgränd på hantavirusets smittoväg. Men nu har man upptäckt flera fall där ett mycket farligt sydamerikanskt hantavirus faktiskt har överförts mellan människor. En misstänkt smittväg är saliven. Detta innebär att hantavirus alltså kan smitta människor som aldrig ens kommit i kontakt med infekterade gnagare. Vi undrade om Puumala-viruset, det hantavirus som finns i Sverige, också kan smitta mellan människor. Vi upptäckte att höga halter av hantavirusets gener faktiskt fanns i saliven hos sorkfeberpatienter. För att undersöka om detta motsvarade smittsamt virus, använde vi salivproverna till att försöka infektera skogssorkar. Eftersom ingen av skogssorkarna blev infekterad verkar det som att patienternas saliv inte innehöll smittsamt virus, trots att virusets gener hade påvisats. Frågan var nu varför?

Kan människans saliv bekämpa hantavirus?

Man har tidigare visat att människans saliv kan bekämpa virus som till exempel HIV och hepatit C virus. Kanske har vi ämnen i saliven som också kan attackera hantaviruset och göra så att det inte kan infektera. Vi visade att saliv från friska försökspersoner faktiskt kunde minska virusets smittsamhet drastiskt. Vi undersökte också olika enskilda ämnen som finns i saliv och fann att det slembildande proteinet mucin var mest effektivt mot hantavirus.

9 ACKNOWLEDGEMENTS

Firstly, I would like to thank all of you who have read this far…

The work behind this thesis was supported by grants from the Swedish Research Council and from the European Union. A lot of people have contributed to the work and I would like to thank all of you, and especially:

Åke Lundkvist, my main supervisor, for inviting me, J2, a "green" biologist, into the group, although I did just have one single virology lesson in my undergraduate days, and that lesson was given by you! For your great knowledge and willingness to share it. For all creative discussions, like when we planned paper I on board your boat! For your encouragement and positive attitude towards my work. Thank you for being there.

Jonas Klingström, J1, my co-supervisor, for always being in such a happy mode and full of new ideas. For always having time to help and for having answers to almost everything and for knowing how to find out the rest. You are the perfect combination of a friend and a supervisor.

Sirkka Vene, for knowing so many things. Without you, a lot of words, sentences and lab material, definitely would be in the wrong place. **Kerstin Falk**, for always telling me, the sometimes uncomfortable, truths about my results, figures and especially choice of colours. **Elisabeth G**, you are quite alright for being a "chromo…". Thanks for the entertainment! All other former and current members of the VZO: **Kirill** (thanks for the tree), **Henrik**, **Gunnel L**, **Angerd**, **Mona**, **Maria W**, **Irina G, Linda B** and **Linda T**, for creating that special VZO-family feeling.

Bråve, for being my room-mate, course-mate, pub-fixing-mate, and pretty-much-everything-mate during these years. **Sara**, for all the fun times in the office, and outside. **Malin S**, for keeping my belgo-beer knowledge updated. **Bobby**, **Susanne** and **Lindvi** for being so nice and helpful colleagues. **Kristian,** for good tips, especially about the CK 18 seminar and **Stig Linder,** for giving it.

My former and present leaders: **Annika Linde**, for the cosy examination, **Anders Tegnell**, **Gunnar Sandström** and **Sören Andersson**. **Johan Struwe** for also becoming an appreciated friend outside work.

Ali Mirazimi for fruitful discussions, **Mikael Nilsson**, for exciting P4-exercises, **Jan Albert**, **Anna-Lena Hammarin**, **Britta Wahren** for creating the atmosphere of knowledge that I have really enjoyed. All other former and current colleagues at virologen, not least **Kajsa**, **Pernilla**, **Margareta B**, **Gunnel E, Dace, Annika K** and **Irina M**.

Clas Ahlm, Lisa Pettersson, Magnus Evander and all other colleagues in Umeå**,** for giving us a new perspective from the north. **Kjell Olof Hedlund** for the new perspective from the basement. **Gert Olsson** for being my favourite ecological alternative. **Eric Rowcliffe** for great days in Korea.

Melinda, for taking care of me in the P4-lab. Did I hear "batteri"?. **Ida A,** for being such a nice friend and for sharing the rare "Fenland loop deficiency syndrome". All other current and former colleagues at the KCB department: **Sándor**, **Marie J**, **Kubby**, **Helen**, **Talar**, **Tara**, **Ralf, Åsa B** and **Sven. Monica Hammarberg**, for excellent help on practical matters, like fixing trips and lending the beetle.

All the young, good-looking students: **David**, **Jolle**, **Anne-Marie**, **Cecilia**, **Karin**, **Carina** and **Sofie** for making me certain that life (and party) goes on.

Andreas Mörner, the friendliest "Mördare" I know. Let´s have some coffee… from a feferoni! **Martin Vondrazek**, for all good advice about parenting, really appreciated.

Anne, **Bartek**, **Kalle**, **Erik**, **Lars E** and **Karin W** and all other nice colleagues from a long time ago.

All friends in Finland and especially **Heikki Henttonnen** for all tricks with rodents and shrews and for letting me be the sauna master for one day. **Olli Vapalahti**, **Alex Plyusnin** and **Antti Vaheri** for your good ideas and nice attitude towards students during the Nordic Zoonosis meetings.

Supporters outside work:
Lotty and **Birgitta** in Tensta: My test is finished. You are the best "öppna förskola"!

The "Spånga-gänget": **Jens**, **Staffan**, **Johan** and **David** for nice evenings in JoJo-Bar and for joining me back in the days, doing "Svanis" and taking the 22:24 train to monkan! **Åsa A** and **Nettan** for joining the party. **Åsa S**, **Anton**, **Pia**, **Ida** and **Andy** for extremely fun "family"-trips.

Karro and **Peter**, we need to plan soon! What were we planning? **Mårten,** my port wine twin and **Annie** the great müslimaker.

Linda I, Jocke, **Linda J, Mange**, **Maria, Frans**, **Lotta, Stefan** and all your wonderful **kids** that keep popping up, for being the best company during vacations, new year parties and other occasions.

Eva and Anders, for all game meat, potatoes and carrot-cakes. **Nisse Holm**, for being my only 90+ friend that I can E-mail.

Ulla (mamma uggla) and Roffe, for being so generous about everything.

Maj-Lis and Leffe, my parents, for always supporting me, and my sometimes, odd projects. **Björn**, my brother and personal IT-support and **Jenny,** for keeping me company in the Larskullen-river.

Albin, my best playmate. I am so happy that you seem to share my interests in beer brewing and playing the did-doo. Your fantastic smile, especially in early mornings, has made it both easier and harder to complete this work.

Malin, for all discussions at work and for all the love at home, and not the other way around. You are the best. “I dig everything”!

So, thank you all. It has been a lot of fun. Can we do it again?

10 REFERENCES

[1] Johnson KM. Hantaviruses: history and overview. Curr Top Microbiol Immunol 2001;256:1-14.

[2] Lee HW. Epidemiology and pathogenesis of hemorrhagic fever with renal syndrome. In: Elliott RM, editor. The Bunyaviridae. New York, Plenum Press, 1996: 253–67.

[3] Lee HW. Hantavirus hunting. Seoul: Sigongsa, 2004.

[4] Paul JR, Mc CW. Epidemic hemorrhagic fever attack rates among United Nations troops during the Korean war. Am J Hyg 1958;68(2):126-39.

[5] Lee HW, Lee PW, Johnson KM. Isolation of the etiologic agent of Korean Hemorrhagic fever. J Infect Dis 1978;137(3):298-308.

[6] White JD, Shirey FG, French GR, Huggins JW, Brand OM, Lee HW. Hantaan virus, aetiological agent of Korean haemorrhagic fever, has Bunyaviridae-like morphology. Lancet 1982;1(8275):768-71.

[7] McCormick JB, Sasso DR, Palmer EL, Kiley MP. Morphological identification of the agent of Korean haemorrhagic fever (Hantaan virus)as a member of the Bunyaviridae. Lancet 1982;1(8275):765-8.

[8] 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.

[9] Brummer-Korvenkontio M, Vaheri A, Hovi T, von Bonsdorff CH, Vuorimies J, Manni 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.

[10] Brummer-Korvenkontio M, Henttonen H, Vaheri A. Hemorrhagic fever with renal syndrome in Finland: ecology and virology of nephropathia epidemica. Scand J Infect Dis Suppl 1982;36:88-91.

[11] Vapalahti O, Mustonen J, Lundkvist A, Henttonen H, Plyusnin A, Vaheri A. Hantavirus infections in Europe. Lancet Infect Dis 2003;3(10):653-61.

[12] Settergren B, Juto P, Trollfors B, Wadell G, Norrby SR. Clinical characteristics of nephropathia epidemica in Sweden: prospective study of 74 cases. Rev Infect Dis 1989;11(6):921-7.

[13] Myhrman G. En njursjukdom med egenartad symptombild. Nord Med Tidsk 1934;7:793-4.

[14] Zetterholm S. Akuta nefriter simulerande akuta bukfall. Läkartidningen 1934;31:425-9.

[15] Juto P, Elgh F, Ahlm C, Alexeyev OA, Edlund K, Lundkvist A, et al. The first human isolate of Puumala virus in Scandinavia as cultured from phytohemagglutinin stimulated leucocytes. J Med Virol 1997;53(2):150-6.

[16] 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.

[17] 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.

[18] Lee PW, Amyx HL, Gajdusek DC, Yanagihara RT, Goldgaber D, Gibbs CJ, Jr. New hemorrhagic fever with renal syndrome-related virus in rodents in the United States. Lancet 1982;2(8312):1405.

[19] Lee PW, Amyx HL, Yanagihara R, Gajdusek DC, Goldgaber D, Gibbs CJ, Jr. Partial characterization of Prospect Hill virus isolated from meadow voles in the United States. J Infect Dis 1985;152(4):826-9.

[20] Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 1993;262(5135):914-7.

[21] Plyusnin A, Morzunov SP. Virus evolution and genetic diversity of hantaviruses and their rodent hosts. Curr Top Microbiol Immunol 2001;256:47-75.

[22] Hughes AL, Friedman R. Evolutionary diversification of protein-coding genes of hantaviruses. Mol Biol Evol 2000;17(10):1558-68.

[23] Khaiboullina SF, Morzunov SP, St Jeor SC. Hantaviruses: molecular biology, evolution and pathogenesis. Curr Mol Med 2005;5(8):773-90.

[24] Gonzalez JP, McCormick JB, Baudon D, Gautun JP, Meunier DY, Dournon E, et al. Serological evidence for Hantaan-related virus in Africa. Lancet 1984;2(8410):1036-7.

[25] Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, et al. Hantavirus in African wood mouse, Guinea. Emerg Infect Dis 2006;12(5):838-40.

[26] Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, et al. Novel hantavirus sequences in Shrew, Guinea. Emerg Infect Dis 2007;13(3):520-2.

[27] Arai S, Bennett SN, Sumibcay L, Cook JA, Song JW, Hope A, 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.

[28] Song JW, Gu SH, Bennett SN, Arai S, Puorger M, Hilbe M, et al. Seewis virus, a genetically distinct hantavirus in the Eurasian common shrew (Sorex araneus). Virol J 2007;4:114.

[29] Song JW, Kang HJ, Song KJ, Truong TT, Bennett SN, Arai S, et al. Newfound hantavirus in Chinese mole shrew, Vietnam. Emerg Infect Dis 2007;13(11):1784-7.

[30] Arai S, Song JW, Sumibcay L, Bennett SN, Nerurkar VR, Parmenter C, et al. Hantavirus in northern short-tailed shrew, United States. Emerg Infect Dis 2007;13(9):1420-3.

[31] 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.

[32] Plyusnin A, Vapalahti O, Vaheri A. Hantaviruses: genome structure, expression and evolution. J Gen Virol 1996;77 (Pt 11):2677-87.

[33] Xiao SY, Leduc JW, Chu YK, Schmaljohn CS. Phylogenetic analyses of virus isolates in the genus Hantavirus, family Bunyaviridae. Virology 1994;198(1):205-17.

[34] Ramsden C, Melo FL, Figueiredo LM, Holmes EC, Zanotto PM. High Rates of Molecular Evolution in Hantaviruses. Mol Biol Evol 2008.

[35] Yashina LN, Patrushev NA, Ivanov LI, Slonova RA, Mishin VP, Kompanez GG, et al. Genetic diversity of hantaviruses associated with hemorrhagic fever with renal syndrome in the far east of Russia. Virus Res 2000;70(1-2):31-44.

[36] Wang H, Yoshimatsu K, Ebihara H, Ogino M, Araki K, Kariwa H, et al. Genetic diversity of hantaviruses isolated in china and characterization of novel hantaviruses isolated from Niviventer confucianus and Rattus rattus. Virology 2000;278(2):332-45.

[37] Avsic-Zupanc T, Toney A, Anderson K, Chu YK, Schmaljohn C. Genetic and antigenic properties of Dobrava virus: a unique member of the Hantavirus genus, family Bunyaviridae. J Gen Virol 1995;76 (Pt 11):2801-8.

[38] 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.

[39] Nemirov K, Vapalahti O, Lundkvist A, Vasilenko V, Golovljova I, Plyusnina A, et al. Isolation and characterization of Dobrava hantavirus carried by the striped field mouse (Apodemus agrarius) in Estonia. J Gen Virol 1999;80 (Pt 2):371-9.

[40] Kariwa H, Yoshizumi S, Arikawa J, Yoshimatsu K, Takahashi K, Takashima I, et al. Evidence for the existence of Puumula-related virus among Clethrionomys rufocanus in Hokkaido, Japan. Am J Trop Med Hyg 1995;53(3):222-7.

[41] Song KJ, Baek LJ, Moon S, Ha SJ, Kim SH, Park KS, 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.

[42] Horling J, Chizhikov V, Lundkvist A, Jonsson M, Ivanov L, Dekonenko 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.

[43] Plyusnin A, Vapalahti O, Lankinen H, Lehvaslaiho H, Apekina N, Myasnikov Y, et al. Tula virus: a newly detected hantavirus carried by European common voles. J Virol 1994;68(12):7833-9.

[44] Vapalahti O, Lundkvist A, Kukkonen SK, Cheng Y, Gilljam M, Kanerva M, et al. Isolation and characterization of Tula virus, a distinct serotype in the genus Hantavirus, family Bunyaviridae. J Gen Virol 1996;77 (Pt 12):3063-7.

[45] Vapalahti O, Lundkvist A, Fedorov V, Conroy CJ, Hirvonen S, Plyusnina A, 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.

[46] Kariwa H, Yoshimatsu K, Sawabe J, Yokota E, Arikawa J, Takashima I, et al. Genetic diversities of hantaviruses among rodents in Hokkaido, Japan and Far East Russia. Virus Res 1999;59(2):219-28.

[47] Hjelle B, Jenison SA, Goade DE, Green WB, Feddersen RM, Scott AA. Hantaviruses: clinical, microbiologic, and epidemiologic aspects. Crit Rev Clin Lab Sci 1995;32(5-6):469-508.

[48] Song W, Torrez-Martinez N, Irwin W, Harrison FJ, Davis R, Ascher M, et al. Isla Vista virus: a genetically novel hantavirus of the California vole Microtus californicus. J Gen Virol 1995;76 (Pt 12):3195-9.

[49] Lopez N, Padula P, Rossi C, Lazaro ME, Franze-Fernandez MT. Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. Virology 1996;220(1):223-6.

[50] Calderon G, Pini N, Bolpe J, Levis S, Mills J, Segura E, et al. Hantavirus reservoir hosts associated with peridomestic habitats in Argentina. Emerg Infect Dis 1999;5(6):792-7.

[51] Johnson AM, de Souza LT, Ferreira IB, Pereira LE, Ksiazek TG, Rollin PE, et al. Genetic investigation of novel hantaviruses causing fatal HPS in Brazil. J Med Virol 1999;59(4):527-35.

[52] Suzuki A, Bisordi I, Levis S, Garcia J, Pereira LE, Souza RP, et al. Identifying rodent hantavirus reservoirs, Brazil. Emerg Infect Dis 2004;10(12):2127-34.

[53] Morzunov SP, Feldmann H, Spiropoulou CF, Semenova VA, Rollin PE, Ksiazek TG, et al. A newly recognized virus associated with a fatal case of hantavirus pulmonary syndrome in Louisiana. J Virol 1995;69(3):1980-3.

[54] Levis S, Morzunov SP, Rowe JE, Enria D, Pini N, Calderon G, et al. Genetic diversity and epidemiology of hantaviruses in Argentina. J Infect Dis 1998;177(3):529-38.

[55] 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.

[56] Rollin PE, Ksiazek TG, Elliott LH, Ravkov EV, Martin ML, Morzunov S, et al. Isolation of black creek canal virus, a new hantavirus from Sigmodon hispidus in Florida. J Med Virol 1995;46(1):35-9.

[57] Ravkov EV, Rollin PE, Ksiazek TG, Peters CJ, Nichol ST. Genetic and serologic analysis of Black Creek Canal virus and its association with human disease and Sigmodon hispidus infection. Virology 1995;210(2):482-9.

[58] 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.

[59] Vincent MJ, Quiroz E, Gracia F, Sanchez AJ, Ksiazek TG, Kitsutani PT, et al. Hantavirus pulmonary syndrome in Panama: identification of novel hantaviruses and their likely reservoirs. Virology 2000;277(1):14-9.

[60] Fulhorst CF, Monroe MC, Salas RA, Duno G, Utrera A, Ksiazek TG, 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.

[61] Milazzo ML, Cajimat MN, Hanson JD, Bradley RD, Quintana M, Sherman C, et al. Catacamas virus, a hantaviral species naturally associated with Oryzomys couesi (Coues' oryzomys) in Honduras. Am J Trop Med Hyg 2006;75(5):1003-10.

[62] Hjelle B, Chavez-Giles F, Torrez-Martinez N, Yates T, Sarisky J, Webb J, et al. Genetic identification of a novel hantavirus of the harvest mouse Reithrodontomys megalotis. J Virol 1994;68(10):6751-4.

[63] Levis S, Garcia J, Pini N, Calderon G, Ramirez J, Bravo D, et al. Hantavirus pulmonary syndrome in northwestern Argentina: circulation of Laguna Negra virus associated with Calomys callosus. Am J Trop Med Hyg 2004;71(5):658-63.

[64] Chu YK, Milligan B, Owen RD, Goodin DG, Jonsson CB. Phylogenetic and geographical relationships of hantavirus strains in eastern and western Paraguay. Am J Trop Med Hyg 2006;75(6):1127-34.

[65] Vasconcelos MI, Lima VP, Iversson LB, Rosa MD, da Rosa AP, da Rosa ES, et al. Hantavirus pulmonary syndrome in the rural area of Juquitiba, Sao Paulo metropolitan area, Brazil. Rev Inst Med Trop Sao Paulo 1997;39(4):237-8.

[66] Johnson AM, Bowen MD, Ksiazek TG, Williams RJ, Bryan RT, Mills JN, et al. Laguna Negra virus associated with HPS in western Paraguay and Bolivia. Virology 1997;238(1):115-27.

[67] Williams RJ, Bryan RT, Mills JN, Palma RE, Vera I, De Velasquez F, et al. An outbreak of hantavirus pulmonary syndrome in western Paraguay. Am J Trop Med Hyg 1997;57(3):274-82.

[68] 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.

[69] 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.

[70] Milazzo ML, Eyzaguirre EJ, Molina CP, Fulhorst CF. Maporal viral infection in the Syrian golden hamster: a model of hantavirus pulmonary syndrome. J Infect Dis 2002;186(10):1390-5.

[71] 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.

[72] Rawlings JA, Torrez-Martinez N, Neill SU, Moore GM, Hicks BN, Pichuantes S, 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.

[73] Hjelle B, Lee SW, Song W, Torrez-Martinez N, Song JW, Yanagihara R, et al. Molecular linkage of hantavirus pulmonary syndrome to the white-footed mouse, Peromyscus leucopus: genetic characterization of the M genome of New York virus. J Virol 1995;69(12):8137-41.

[74] Song JW, Baek LJ, Gajdusek DC, Yanagihara R, Gavrilovskaya I, Luft BJ, et al. Isolation of pathogenic hantavirus from white-footed mouse (Peromyscus leucopus). Lancet 1994;344(8937):1637.

[75] Chu YK, Owen RD, Sanchez-Hernandez C, Romero-Almaraz Mde L, Jonsson CB. Genetic characterization and phylogeny of a hantavirus from Western Mexico. Virus Res 2008;131(2):180-8.

[76] Hjelle B, Torrez-Martinez N, Koster FT. Hantavirus pulmonary syndrome-related virus from Bolivia. Lancet 1996;347(8993):57.

[77] 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.

[78] 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.

[79] Elliott LH, Ksiazek TG, Rollin PE, Spiropoulou CF, Morzunov S, Monroe M, et al. Isolation of the causative agent of hantavirus pulmonary syndrome. Am J Trop Med Hyg 1994;51(1):102-8.

[80] Olsson GE, Dalerum F, Hornfeldt B, Elgh F, Palo TR, Juto P, et al. Human hantavirus infections, Sweden. Emerg Infect Dis 2003;9(11):1395-401.

[81] 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.

[82] 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.

[83] Olsson GE, Ahlm C, Elgh F, Verlemyr AC, White N, Juto P, et al. Hantavirus antibody occurrence in bank voles (Clethrionomys glareolus) during a vole population cycle. J Wildl Dis 2003;39(2):299-305.

[84] Statistik för sorkfeber. In: http://www.smittskyddsinstitutet.se/statistik/sorkfeber/. Homepage, 2008.

[85] Pettersson L BJ, Juto P, Evander M, Ahlm C. Outbreak of Puumala virus infection, Sweden. Emerg Infect Dis 2008;[Epub ahead of print].

[86] Goldsmith CS, Elliott LH, Peters CJ, Zaki SR. Ultrastructural characteristics of Sin Nombre virus, causative agent of hantavirus pulmonary syndrome. Arch Virol 1995;140(12):2107-22.

[87] Jaaskelainen KM, Kaukinen P, Minskaya ES, Plyusnina A, Vapalahti O, Elliott RM, et al. Tula and Puumala hantavirus NSs ORFs are functional and the products inhibit activation of the interferon-beta promoter. J Med Virol 2007;79(10):1527-36.

[88] Gavrilovskaya IN, Brown EJ, Ginsberg MH, Mackow ER. Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. J Virol 1999;73(5):3951-9.

[89] Gavrilovskaya IN, Shepley M, Shaw R, Ginsberg MH, Mackow ER. beta3 Integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc Natl Acad Sci U S A 1998;95(12):7074-9.

[90] Krautkramer E, Zeier M. Hantavirus causing hemorrhagic fever with renal syndrome enters from the apical surface and requires decay-accelerating factor (DAF/CD55). J Virol 2008;82(9):4257-64.

[91] Jin M, Park J, Lee S, Park B, Shin J, Song KJ, et al. Hantaan virus enters cells by clathrin-dependent receptor-mediated endocytosis. Virology 2002;294(1):60-9.

[92] Dunn EF, Pritlove DC, Jin H, Elliott RM. Transcription of a recombinant bunyavirus RNA template by transiently expressed bunyavirus proteins. Virology 1995;211(1):133-43.

[93] Garcin D, Lezzi M, Dobbs M, Elliott RM, Schmaljohn C, Kang CY, et al. The 5' ends of Hantaan virus (Bunyaviridae) RNAs suggest a prime-and-realign mechanism for the initiation of RNA synthesis. J Virol 1995;69(9):5754-62.

[94] Krug RM. Priming of influenza viral RNA transcription by capped heterologous RNAs. Curr Top Microbiol Immunol 1981;93:125-49.

[95] Jonsson CB, Schmaljohn CS. Replication of hantaviruses. Curr Top Microbiol Immunol 2001;256:15-32.

[96] Lober C, Anheier B, Lindow S, Klenk HD, Feldmann H. The Hantaan virus glycoprotein precursor is cleaved at the conserved pentapeptide WAASA. Virology 2001;289(2):224-9.

[97] Antic D, Wright KE, Kang CY. Maturation of Hantaan virus glycoproteins G1 and G2. Virology 1992;189(1):324-8.

[98] Ruusala A, Persson R, Schmaljohn CS, Pettersson RF. Coexpression of the membrane glycoproteins G1 and G2 of Hantaan virus is required for targeting to the Golgi complex. Virology 1992;186(1):53-64.

[99] Pensiero MN, Jennings GB, Schmaljohn CS, Hay J. Expression of the Hantaan virus M genome segment by using a vaccinia virus recombinant. J Virol 1988;62(3):696-702.

[100] Spiropoulou CF. Hantavirus maturation. Curr Top Microbiol Immunol 2001;256:33-46.

[101] Pettersson R.F. ML. Synthesis, assembly and intracellular transport of Bunyaviridae membrane proteins. In: Elliott LH, editor. The Bunyaviridae. New York, Plenum Press, 1996: 159–88.

[102] Ravkov EV, Nichol ST, Compans RW. Polarized entry and release in epithelial cells of Black Creek Canal virus, a New World hantavirus. J Virol 1997;71(2):1147-54.

[103] Ulrich R, Hjelle B, Pitra C, Kruger DH. Emerging viruses: the case 'hantavirus'. Intervirology 2002;45(4-6):318-27.

[104] Maes P, Clement J, Gavrilovskaya I, Van Ranst M. Hantaviruses: immunology, treatment, and prevention. Viral Immunol 2004;17(4):481-97.

[105] Lee JS. Clinical manifestations and treatment of HFRS and HPS. In: Lee HW, Calisher CH, Schmaljohn C, editors. Manual of hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. Seoul, WHO Collaborating Center for Virus Reference and Research, 1998: 18–27.

[106] Yanagihara R, Silverman DJ. Experimental infection of human vascular endothelial cells by pathogenic and nonpathogenic hantaviruses. Arch Virol 1990;111(3-4):281-6.

[107] Cosgriff TM. Mechanisms of disease in Hantavirus infection: pathophysiology of hemorrhagic fever with renal syndrome. Rev Infect Dis 1991;13(1):97-107.

[108] Peters CJ, Simpson GL, Levy H. Spectrum of hantavirus infection: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. Annu Rev Med 1999;50:531-45.

[109] Terajima M, Vapalahti O, Van Epps HL, Vaheri A, Ennis FA. Immune responses to Puumala virus infection and the pathogenesis of nephropathia epidemica. Microbes Infect 2004;6(2):238-45.

[110] Ahlm C, Juto P, Stegmayr B, Settergren B, Wadell G, Tarnvik A, et al. Prevalence of serum antibodies to hantaviruses in northern Sweden as measured by recombinant nucleocapsid proteins. Scand J Infect Dis 1997;29(4):349-54.

[111] Case Information: Hantavirus Pulmonary Syndrome Case Count and Descriptive Statistics. In, http://www.cdc.gov/ncidod/diseases/hanta/hps/noframes/caseinfo.htm. Homepage, 2008.

[112] Vial PA, Valdivieso F, Mertz G, Castillo C, Belmar E, Delgado I, et al. Incubation period of hantavirus cardiopulmonary syndrome. Emerg Infect Dis 2006;12(8):1271-3.

[113] Enria DA, Briggiler AM, Pini N, Levis S. Clinical manifestations of New World hantaviruses. Curr Top Microbiol Immunol 2001;256:117-34.

[114] Kirsi JJ, North JA, McKernan PA, Murray BK, Canonico PG, Huggins JW, et al. Broad-spectrum antiviral activity of 2-beta-D-ribofuranosylselenazole-4-carboxamide, a new antiviral agent. Antimicrob Agents Chemother 1983;24(3):353-61.

[115] Huggins JW, Kim GR, Brand OM, McKee KT, Jr. Ribavirin therapy for Hantaan virus infection in suckling mice. J Infect Dis 1986;153(3):489-97.

[116] Huggins JW, Hsiang CM, Cosgriff TM, Guang MY, Smith JI, Wu ZO, et al. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. J Infect Dis 1991;164(6):1119-27.

[117] Chapman LE, Mertz GJ, Peters CJ, Jolson HM, Khan AS, Ksiazek TG, et al. Intravenous ribavirin for hantavirus pulmonary syndrome: safety and tolerance during 1 year of open-label experience. Ribavirin Study Group. Antivir Ther 1999;4(4):211-9.

[118] Cho HW, Howard CR. Antibody responses in humans to an inactivated hantavirus vaccine (Hantavax). Vaccine 1999;17(20-21):2569-75.

[119] Krüger DH, Ulrich R, Lundkvist Å. Hantavirus infections and their prevention. Microbes infect 2001;3:1129-44.

[120] Cho HW, Howard CR, Lee HW. Review of an inactivated vaccine against hantaviruses. Intervirology 2002;45(4-6):328-33.

[121] Hjelle B. Vaccines against hantaviruses. Expert Rev Vaccines 2002;1(3):373-84.

[122] Song G, Huang YC, Hang CS, Hao FY, Li DX, Zheng XL, et al. Preliminary human trial of inactivated golden hamster kidney cell (GHKC) vaccine against haemorrhagic fever with renal syndrome (HFRS). Vaccine 1992;10(4):214-6.

[123] Lu Q, Zhu Z, Weng J. Immune responses to inactivated vaccine in people naturally infected with hantaviruses. J Med Virol 1996;49(4):333-5.

[124] Hooper JW, Li D. Vaccines against hantaviruses. Curr Top Microbiol Immunol 2001;256:171-91.

[125] Ye C, Prescott J, Nofchissey R, Goade D, Hjelle B. Neutralizing antibodies and Sin Nombre virus RNA after recovery from hantavirus cardiopulmonary syndrome. Emerg Infect Dis 2004;10(3):478-82.

[126] Mertz GJ, Hjelle B, Crowley M, Iwamoto G, Tomicic V, Vial PA. Diagnosis and treatment of new world hantavirus infections. Curr Opin Infect Dis 2006;19(5):437-42.

[127] Klingstrom J, Stoltz M, Hardestam J, Ahlm C, Lundkvist A. Passive immunization protects cynomolgus macaques against Puumala hantavirus challenge. Antivir Ther 2008;13(1):125-33.

[128] Hooper JW, Ferro AM, Wahl-Jensen V. Immune serum produced by DNA vaccination protects hamsters against lethal respiratory challenge with Andes virus. J Virol 2008;82(3):1332-8.

[129] Glass GE, Johnson JS, Hodenbach GA, Disalvo CL, Peters CJ, Childs JE, et al. Experimental evaluation of rodent exclusion methods to reduce hantavirus transmission to humans in rural housing. Am J Trop Med Hyg 1997;56(4):359-64.

[130] 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.

[131] Mills JN, Corneli A, Young JC, Garrison LE, Khan AS, Ksiazek TG. Hantavirus pulmonary syndrome--United States: updated recommendations for risk reduction. Centers for Disease Control and Prevention. MMWR Recomm Rep 2002;51(RR-9):1-12.

[132] Ahlm C, Wallin K, Lundkvist A, Elgh F, Juto P, Merza M, et al. Serologic evidence of Puumala virus infection in wild moose in northern Sweden. Am J Trop Med Hyg 2000;62(1):106-11.

[133] Zeier M, Handermann M, Bahr U, Rensch B, Muller S, Kehm R, et al. New ecological aspects of hantavirus infection: a change of a paradigm and a challenge of prevention--a review. Virus Genes 2005;30(2):157-80.

[134] Yang ZQ, Yu SY, Nie J, Chen Q, Li ZF, Liu YX, et al. [Prevalence of hemorrhagic fever with renal syndrome virus in domestic pigs: an epidemiological investigation in Shandong province]. Di Yi Jun Yi Da Xue Xue Bao 2004;24(11):1283-6.

[135] Klein SL. Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. Physiol Behav 2003;79(3):441-9.

[136] Adamo SA. Modulating the modulators: parasites, neuromodulators and host behavioral change. Brain Behav Evol 2002;60(6):370-7.

[137] Lagrue C, Kaldonski N, Perrot-Minnot MJ, Motreuil S, Bollache L. Modification of hosts' behavior by a parasite: field evidence for adaptive manipulation. Ecology 2007;88(11):2839-47.

[138] Klein S, Zink MC, Glass G. Seoul virus infection increases aggressive behaviour in male Norway rats. Animal behaviour 2004;67(3):421-9.

[139] Nuzum EO, Rossi CA, Stephenson EH, LeDuc JW. Aerosol transmission of Hantaan and related viruses to laboratory rats. Am J Trop Med Hyg 1988;38(3):636-40.

[140] 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.

[141] 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.

[142] Calisher CH, Sweeney W, Mills JN, Beaty BJ. Natural history of Sin Nombre virus in western Colorado. Emerg Infect Dis 1999;5(1):126-34.

[143] Mills JN, Ksiazek TG, Ellis BA, Rollin PE, Nichol ST, Yates TL, 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.

[144] Meyer BJ, Schmaljohn CS. Persistent hantavirus infections: characteristics and mechanisms. Trends Microbiol 2000;8(2):61-7.

[145] Klein SL, Calisher CH. Emergence and persistence of hantaviruses. Curr Top Microbiol Immunol 2007;315:217-52.

[146] Hjelle B, Yates T. Modeling hantavirus maintenance and transmission in rodent communities. Curr Top Microbiol Immunol 2001;256:77-90.

[147] Botten J, Mirowsky K, Kusewitt D, Bharadwaj M, Yee J, Ricci R, et al. Experimental infection model for Sin Nombre hantavirus in the deer mouse (Peromyscus maniculatus). Proc Natl Acad Sci U S A 2000;97(19):10578-83.

[148] Padula P, Figueroa R, Navarrete M, Pizarro E, Cadiz R, Bellomo C, et al. Transmission study of Andes hantavirus infection in wild sigmodontine rodents. J Virol 2004;78(21):11972-9.

[149] Klein SL. The effects of hormones on sex differences in infection: from genes to behavior. Neurosci Biobehav Rev 2000;24(6):627-38.

[150] Klein SL, Bird BH, Glass GE. Sex differences in Seoul virus infection are not related to adult sex steroid concentrations in Norway rats. J Virol 2000;74(17):8213-7.

[151] Zuk M, McKean KA. Sex differences in parasite infections: patterns and processes. Int J Parasitol 1996;26(10):1009-23.

[152] Klein SL, Bird BH, Glass GE. Sex differences in immune responses and viral shedding following Seoul virus infection in Norway rats. Am J Trop Med Hyg 2001;65(1):57-63.

[153] Klein S. Host factors mediating sex differences in viral infection. Gend Med 2005;2(4):197-207.

[154] Otteson EW, Riolo J, Rowe JE, Nichol ST, Ksiazek TG, Rollin PE, et al. Occurrence of hantavirus within the rodent population of northeastern California and Nevada. Am J Trop Med Hyg 1996;54(2):127-33.

[155] 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.

[156] Mills JN, Schmidt K, Ellis BA, Calderon G, Enria DA, Ksiazek TG. A longitudinal study of hantavirus infection in three sympatric reservoir species in agroecosystems on the Argentine Pampa. Vector Borne Zoonotic Dis 2007;7(2):229-40.

[157] Kallio ER, Voutilainen L, Vapalahti O, Vaheri A, Henttonen H, Koskela E, et al. Endemic hantavirus infection impairs the winter survival of its rodent host. Ecology 2007;88(8):1911-6.

[158] Olsson GE, White N, Ahlm C, Elgh F, Verlemyr AC, Juto P, et al. Demographic factors associated with hantavirus infection in bank voles (Clethrionomys glareolus). Emerg Infect Dis 2002;8(9):924-9.

[159] Bernshtein AD, Apekina NS, Mikhailova TV, Myasnikov YA, Khlyap LA, Korotkov YS, et al. Dynamics of Puumala hantavirus infection in naturally infected bank voles (Clethrinomys glareolus). Arch Virol 1999;144(12):2415-28.

[160] Childs JE, Glass GE, Korch GW, LeDuc JW. Prospective seroepidemiology of hantaviruses and population dynamics of small mammal communities of Baltimore, Maryland. Am J Trop Med Hyg 1987;37(3):648-62.

[161] Dohmae K, Koshimizu U, Nishimune Y. In utero and mammary transfer of hantavirus antibody from dams to infant rats. Lab Anim Sci 1993;43(6):557-61.

[162] Dohmae K, Nishimune Y. Protection against hantavirus infection by dam's immunity transferred vertically to neonates. Arch Virol 1995;140(1):165-72.

[163] Kallio ER, Poikonen A, Vaheri A, Vapalahti O, Henttonen H, Koskela E, et al. Maternal antibodies postpone hantavirus infection and enhance individual breeding success. Proc Biol Sci 2006;273(1602):2771-6.

[164] Easterbrook JD, Zink MC, Klein SL. Regulatory T cells enhance persistence of the zoonotic pathogen Seoul virus in its reservoir host. Proc Natl Acad Sci U S A 2007;104(39):15502-7.

[165] Schountz T, Prescott J, Cogswell AC, Oko L, Mirowsky-Garcia K, Galvez AP, et al. Regulatory T cell-like responses in deer mice persistently infected with Sin Nombre virus. Proc Natl Acad Sci U S A 2007;104(39):15496-501.

[166] Gavrilovskaya IN, Apekina NS, Myasnikov Yu A, Bernshtein AD, Ryltseva EV, Gorbachkova EA, 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.

[167] Ahlm C, Alexeyev OA, Elgh F, Aava B, Wadell G, Tarnvik A, et al. 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 1997;56(6):674-8.

[168] Madhav NK, Wagoner KD, Douglass RJ, Mills JN. Delayed density-dependent prevalence of Sin Nombre virus antibody in Montana deer mice (Peromyscus maniculatus) and implications for human disease risk. Vector Borne Zoonotic Dis 2007;7(3):353-64.

[169] Mills JN, Ksiazek TG, Peters CJ, Childs JE. Long-term studies of hantavirus reservoir populations in the southwestern United States: a synthesis. Emerg Infect Dis 1999;5(1):135-42.

[170] Abbott KD, Ksiazek TG, Mills JN. Long-term hantavirus persistence in rodent populations in central Arizona. Emerg Infect Dis 1999;5(1):102-12.

[171] Douglass RJ, Wilson T, Semmens WJ, Zanto SN, Bond CW, Van Horn RC, et al. Longitudinal studies of Sin Nombre virus in deer mouse-dominated ecosystems of Montana. Am J Trop Med Hyg 2001;65(1):33-41.

[172] Boone JD, McGwire KC, Otteson EW, DeBaca RS, Kuhn EA, Villard P, et al. Remote sensing and geographic information systems: charting Sin Nombre virus infections in deer mice. Emerg Infect Dis 2000;6(3):248-58.

[173] Yan L, Fang LQ, Huang HG, Zhang LQ, Feng D, Zhao WJ, et al. Landscape elements and Hantaan virus-related hemorrhagic fever with renal syndrome, People's Republic of China. Emerg Infect Dis 2007;13(9):1301-6.

[174] Linard C, Lamarque P, Heyman P, Ducoffre G, Luyasu V, Tersago K, et al. Determinants of the geographic distribution of Puumala virus and Lyme borreliosis infections in Belgium. Int J Health Geogr 2007;6:15.

[175] Yanagihara R, Amyx HL, Gajdusek DC. Experimental infection with Puumala virus, the etiologic agent of nephropathia epidemica, in bank voles (Clethrionomys glareolus). J Virol 1985;55(1):34-8.

[176] Botten J, Mirowsky K, Ye C, Gottlieb K, Saavedra M, Ponce L, et al. Shedding and intracage transmission of Sin Nombre hantavirus in the deer mouse (Peromyscus maniculatus) model. J Virol 2002;76(15):7587-94.

[177] Lee HW, Lee PW, Baek LJ, Song CK, Seong IW. Intraspecific transmission of Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the rodent Apodemus agrarius. Am J Trop Med Hyg 1981;30(5):1106-12.

[178] Botten J, Mirowsky K, Kusewitt D, Ye C, Gottlieb K, Prescott J, et al. Persistent Sin Nombre virus infection in the deer mouse (Peromyscus maniculatus) model: sites of replication and strand-specific expression. J Virol 2003;77(2):1540-50.

[179] Kuenzi AJ, Douglass RJ, Bond CW, Calisher CH, Mills JN. Long-term dynamics of Sin Nombre viral RNA and antibody in deer mice in Montana. J Wildl Dis 2005;41(3):473-81.

[180] Hutchinson KL, Rollin PE, Peters CJ. Pathogenesis of a North American hantavirus, Black Creek Canal virus, in experimentally infected Sigmodon hispidus. Am J Trop Med Hyg 1998;59(1):58-65.

[181] 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(2):365-74.

[182] Safronetz D, Drebot MA, Artsob H, Cote T, Makowski K, Lindsay LR. Sin Nombre Virus shedding patterns in naturally infected deer mice (Peromyscus maniculatus) in relation to duration of infection. Vector Borne Zoonotic Dis 2008;8(1):97-100.

[183] Arnal C, Crance JM, Gantzer C, Schwartzbrod L, Deloince R, Billaudel S. Persistence of infectious hepatitis A virus and its genome in artificial seawater. Zentralbl Hyg Umweltmed 1998;201(3):279-84.

[184] Mbithi JN, Springthorpe VS, Sattar SA. Effect of relative humidity and air temperature on survival of hepatitis A virus on environmental surfaces. Appl Environ Microbiol 1991;57(5):1394-9.

[185] Stallknecht DE, Kearney MT, Shane SM, Zwank PJ. Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. Avian Dis 1990;34(2):412-8.

[186] Abad FX, Villena C, Guix S, Caballero S, Pinto RM, Bosch A. Potential role of fomites in the vehicular transmission of human astroviruses. Appl Environ Microbiol 2001;67(9):3904-7.

[187] 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.

[188] Sauvage F, Langlais M, Yoccoz N, Pontier D. Modelling hantavirus in fluctuating populations of bank voles: the role of indirect transmission on virus persistence. Journal of Animal Ecology 2003;72(1):1-13.

[189] Hurst CJ, Gerba CP, Cech I. Effects of environmental variables and soil characteristics on virus survival in soil. Appl Environ Microbiol 1980;40(6):1067-79.

[190] Lytle CD, Sagripanti JL. Predicted inactivation of viruses of relevance to biodefense by solar radiation. J Virol 2005;79(22):14244-52.

[191] Kallio ER, Klingstrom J, Gustafsson E, Manni T, Vaheri A, Henttonen H, 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.

[192] Linard C, Tersago K, Leirs H, Lambin EF. Environmental conditions and Puumala virus transmission in Belgium. Int J Health Geogr 2007;6:55.

[193] St Jeor SC. Three-week incubation period for hantavirus infection. Pediatr Infect Dis J 2004;23(10):974-5.

[194] Kawamata J, Yamanouchi T, Dohmae K, Miyamoto H, Takahaski M, Yamanishi K, et al. Control of laboratory acquired hemorrhagic fever with renal syndrome (HFRS) in Japan. Lab Anim Sci 1987;37(4):431-6.

[195] Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. Emerg Infect Dis 1997;3(2):95-104.

[196] Vapalahti K, Paunio M, Brummer-Korvenkontio M, Vaheri A, Vapalahti O. Puumala virus infections in Finland: increased occupational risk for farmers. Am J Epidemiol 1999;149(12):1142-51.

[197] Abu Sin M, Stark K, van Treeck U, Dieckmann H, Uphoff H, Hautmann W, et al. Risk factors for hantavirus infection in Germany, 2005. Emerg Infect Dis 2007;13(9):1364-6.

[198] Lundkvist A, Vapalahti O, Henttonen H, Vaheri A, Plyusnin A. Hantavirus infections among mammalogists studied by focus reduction neutralisation test. Eur J Clin Microbiol Infect Dis 2000;19(10):802-3.

[199] Moll van Charante AW, Groen J, Mulder PG, Rijpkema SG, Osterhaus AD. Occupational risks of zoonotic infections in Dutch forestry workers and muskrat catchers. Eur J Epidemiol 1998;14(2):109-16.

[200] Ahlm C, Thelin A, Elgh F, Juto P, Stiernstrom EL, Holmberg S, et al. Prevalence of antibodies specific to Puumala virus among farmers in Sweden. Scand J Work Environ Health 1998;24(2):104-8.

[201] Fritz CL, Fulhorst CF, Enge B, Winthrop KL, Glaser CA, Vugia DJ. Exposure to rodents and rodent-borne viruses among persons with elevated occupational risk. J Occup Environ Med 2002;44(10):962-7.

[202] Vitek CR, Ksiazek TG, Peters CJ, Breiman RF. Evidence against infection with hantaviruses among forest and park workers in the southwestern United States. Clin Infect Dis 1996;23(2):283-5.

[203] Fulhorst CF, Milazzo ML, Armstrong LR, Childs JE, Rollin PE, Khabbaz R, et al. Hantavirus and arenavirus antibodies in persons with occupational rodent exposure. Emerg Infect Dis 2007;13(4):532-8.

[204] Roizman B, Pellett PE. The family Herpesviridae: A brief introduction. In: Knipe DM, Howley PM, editors. Fields' virology. Philadelphia, Lippincott Williams & Wilkins, 2001: 2381-97.

[205] Klingstrom J, Plyusnin A, Vaheri A, Lundkvist A. Wild-type Puumala hantavirus infection induces cytokines, C-reactive protein, creatinine, and nitric oxide in cynomolgus macaques. J Virol 2002;76(1):444-9.

[206] Klingstrom J, Falk KI, Lundkvist A. Delayed viremia and antibody responses in Puumala hantavirus challenged passively immunized cynomolgus macaques. Arch Virol 2005;150(1):79-92.

[207] Hooper JW, Larsen T, Custer DM, Schmaljohn CS. A lethal disease model for hantavirus pulmonary syndrome. Virology 2001;289(1):6-14.

[208] Netski D, Thran BH, St Jeor SC. Sin Nombre virus pathogenesis in Peromyscus maniculatus. J Virol 1999;73(1):585-91.

[209] 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.

[210] Pensiero MN, Sharefkin JB, Dieffenbach CW, Hay J. Hantaan virus infection of human endothelial cells. J Virol 1992;66(10):5929-36.

[211] Peters CJ, Zaki SR. Role of the endothelium in viral hemorrhagic fevers. Crit Care Med 2002;30(5 Suppl):S268-73.

[212] Khaiboullina SF, Netski DM, Krumpe P, St Jeor SC. Effects of tumor necrosis factor alpha on sin nombre virus infection in vitro. J Virol 2000;74(24):11966-71.

[213] Nolte KB, Feddersen RM, Foucar K, Zaki SR, Koster FT, Madar D, et al. Hantavirus pulmonary syndrome in the United States: a pathological description of a disease caused by a new agent. Hum Pathol 1995;26(1):110-20.

[214] Rowe RK, Pekosz A. Bidirectional virus secretion and nonciliated cell tropism following Andes virus infection of primary airway epithelial cell cultures. J Virol 2006;80(3):1087-97.

[215] Sundstrom JB, McMullan LK, Spiropoulou CF, Hooper WC, Ansari AA, Peters CJ, et al. Hantavirus infection induces the expression of RANTES and IP-10 without causing increased permeability in human lung microvascular endothelial cells. J Virol 2001;75(13):6070-85.

[216] Temonen M, Vapalahti O, Holthofer H, Brummer-Korvenkontio M, Vaheri A, Lankinen H. Susceptibility of human cells to Puumala virus infection. J Gen Virol 1993;74 (Pt 3):515-8.

[217] Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foucar K, et al. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am J Pathol 1995;146(3):552-79.

[218] Khaiboullina SF, St Jeor SC. Hantavirus immunology. Viral Immunol 2002;15(4):609-25.

[219] Courouble P, Vanpee D, Delgrange E, Donckier J, Pochet JM, Gillet JB. Hantavirus infections: clinical presentation in the emergency room. Eur J Emerg Med 2001;8(1):17-20.

[220] Duchin JS, Koster FT, Peters CJ, Simpson GL, Tempest B, Zaki SR, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. The Hantavirus Study Group. N Engl J Med 1994;330(14):949-55.

[221] Verity R, Prasad E, Grimsrud K, Artsob H, Drebot M, Miedzinski L, et al. Hantavirus pulmonary syndrome in northern Alberta, Canada: clinical and laboratory findings for 19 cases. Clin Infect Dis 2000;31(4):942-6.

[222] Geimonen E, Neff S, Raymond T, Kocer SS, Gavrilovskaya IN, Mackow ER. Pathogenic and nonpathogenic hantaviruses differentially regulate endothelial cell responses. Proc Natl Acad Sci U S A 2002;99(21):13837-42.

[223] Kraus AA, Raftery MJ, Giese T, Ulrich R, Zawatzky R, Hippenstiel S, et al. Differential antiviral response of endothelial cells after infection with pathogenic and nonpathogenic hantaviruses. J Virol 2004;78(12):6143-50.

[224] Mackow ER, Gavrilovskaya IN. Cellular receptors and hantavirus pathogenesis. Curr Top Microbiol Immunol 2001;256:91-115.

[225] Gavrilovskaya IN, Peresleni T, Geimonen E, Mackow ER. Pathogenic hantaviruses selectively inhibit beta3 integrin directed endothelial cell migration. Arch Virol 2002;147(10):1913-31.

[226] Gavrilovskaya IN, Gorbunova EE, Mackow NA, Mackow ER. Hantaviruses Direct Endothelial Cell Permeability by Sensitizing Cells to the Vascular Permeability Factor, VEGF, while Angiopoietin-1 and Sphingosine 1-Phosphate inhibit Hantavirus-directed Permeability. J Virol 2008.

[227] Kato H, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K, et al. Cell type-specific involvement of RIG-I in antiviral response. Immunity 2005;23(1):19-28.

[228] Davis IC, Zajac AJ, Nolte KB, Botten J, Hjelle B, Matalon S. Elevated generation of reactive oxygen/nitrogen species in hantavirus cardiopulmonary syndrome. J Virol 2002;76(16):8347-59.

[229] Tamura M, Asada H, Kondo K, Takahashi M, Yamanishi K. Effects of human and murine interferons against hemorrhagic fever with renal syndrome (HFRS) virus (Hantaan virus). Antiviral Res 1987;8(4):171-8.

[230] Klingstrom J, Akerstrom S, Hardestam J, Stoltz M, Simon M, Falk KI, et al. Nitric oxide and peroxynitrite have different antiviral effects against hantavirus replication and free mature virions. Eur J Immunol 2006;36(10):2649-57.

[231] Frese M, Kochs G, Feldmann H, Hertkorn C, Haller O. Inhibition of bunyaviruses, phleboviruses, and hantaviruses by human MxA protein. J Virol 1996;70(2):915-23.

[232] Kanerva M, Melen K, Vaheri A, Julkunen I. Inhibition of puumala and tula hantaviruses in Vero cells by MxA protein. Virology 1996;224(1):55-62.

[233] Alff PJ, Gavrilovskaya IN, Gorbunova E, Endriss K, Chong Y, Geimonen E, et al. The pathogenic NY-1 hantavirus G1 cytoplasmic tail inhibits RIG-I- and TBK-1-directed interferon responses. J Virol 2006;80(19):9676-86.

[234] Stoltz M, Ahlm C, Lundkvist A, Klingstrom J. Lambda interferon (IFN-lambda) in serum is decreased in hantavirus-infected patients, and in vitro-established infection is insensitive to treatment with all IFNs and inhibits IFN-gamma-induced nitric oxide production. J Virol 2007;81(16):8685-91.

[235] Mori M, Rothman AL, Kurane I, Montoya JM, Nolte KB, Norman JE, et al. High levels of cytokine-producing cells in the lung tissues of patients with fatal hantavirus pulmonary syndrome. J Infect Dis 1999;179(2):295-302.

[236] Linderholm M, Ahlm C, Settergren B, Waage A, Tarnvik A. Elevated plasma levels of tumor necrosis factor (TNF)-alpha, soluble TNF receptors, interleukin (IL)-6, and IL-10 in patients with hemorrhagic fever with renal syndrome. J Infect Dis 1996;173(1):38-43.

[237] Kanerva M, Mustonen J, Vaheri A. Pathogenesis of puumala and other hantavirus infections. Rev Med Virol 1998;8(2):67-86.

[238] Kanerva M, Vaheri A, Mustonen J, Partanen J. High-producer allele of tumour necrosis factor-alpha is part of the susceptibility MHC haplotype in severe puumala virus-induced nephropathia epidemica. Scand J Infect Dis 1998;30(5):532-4.

[239] Raftery MJ, Kraus AA, Ulrich R, Kruger DH, Schonrich G. Hantavirus infection of dendritic cells. J Virol 2002;76(21):10724-33.

[240] Klingstrom J, Lindgren T, Ahlm C. Sex-dependent differences in plasma cytokine responses to hantavirus infection. Clin Vaccine Immunol 2008;15(5):885-7.

[241] Groen J, Gerding M, Jordans JG, Clement JP, Osterhaus AD. Class and subclass distribution of Hantavirus-specific serum antibodies at different times after the onset of nephropathia epidemica. J Med Virol 1994;43(1):39-43.

[242] Settergren B, Ahlm C, Juto P, Niklasson B. Specific Puumala IgG virus half a century after haemorrhagic fever with renal syndrome. Lancet 1991;338(8758):66.

[243] 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.

[244] de Carvalho Nicacio C, Bjorling E, Lundkvist A. Immunoglobulin A responses to Puumala hantavirus. J Gen Virol 2000;81(Pt 6):1453-61.

[245] Evander M, Eriksson I, Pettersson L, Juto P, Ahlm C, Olsson GE, et al. Puumala hantavirus viremia diagnosed by real-time reverse transcriptase PCR using samples from patients with hemorrhagic fever and renal syndrome. J Clin Microbiol 2007;45(8):2491-7.

[246] Plyusnin A, Horling J, Kanerva M, Mustonen J, Cheng Y, Partanen J, 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.

[247] Plyusnin A, Mustonen J, Asikainen K, Plyusnina A, Niemimaa J, Henttonen H, et al. Analysis of puumala hantavirus genome in patients with nephropathia epidemica and rodent carriers from the sites of infection. J Med Virol 1999;59(3):397-405.

[248] Van Epps HL, Terajima M, Mustonen J, Arstila TP, Corey EA, Vaheri A, et al. Long-lived memory T lymphocyte responses after hantavirus infection. J Exp Med 2002;196(5):579-88.

[249] Elgh F, Wadell G, Juto P. Comparison of the kinetics of Puumala virus specific IgM and IgG antibody responses in nephropathia epidemica as measured by a recombinant antigen-based enzyme-linked immunosorbent assay and an immunofluorescence test. J Med Virol 1995;45(2):146-50.

[250] Groen J, Dalrymple J, Fisher-Hoch S, Jordans JG, Clement JP, Osterhaus AD. Serum antibodies to structural proteins of Hantavirus arise at different times after infection. J Med Virol 1992;37(4):283-7.

[251] Wang ML. Production of IgA monoclonal antibodies against influenza A virus. J Virol Methods 1986;13(1):21-6.

[252] Mazanec MB, Nedrud JG, Lamm ME. Immunoglobulin A monoclonal antibodies protect against Sendai virus. J Virol 1987;61(8):2624-6.

[253] Alexeyev OA, Ahlm C, Billheden J, Settergren B, Wadell G, Juto P. Elevated levels of total and Puumala virus-specific immunoglobulin E in the Scandinavian type of hemorrhagic fever with renal syndrome. Clin Diagn Lab Immunol 1994;1(3):269-72.

[254] Alexeyev OA. Two cases of hyperimmunoglobulinemia E in patients with hemorrhagic fever with renal syndrome. Scand J Infect Dis 1991;23(3):391.

[255] Markotic A, Dasic G, Gagro A, Sabioncello A, Rabatic S, Kuzman I, et al. Role of peripheral blood mononuclear cell (PBMC) phenotype changes in the pathogenesis of haemorrhagic fever with renal syndrome (HFRS). Clin Exp Immunol 1999;115(2):329-34.

[256] Alexeyev OA, Linderholm M, Elgh F, Wadell G, Juto P, Tarnvik A. Increased plasma levels of soluble CD23 in haemorrhagic fever with renal syndrome; relation to virus-specific IgE. Clin Exp Immunol 1997;109(2):351-5.

[257] Borish L, Mascali JJ, Rosenwasser LJ. IgE-dependent cytokine production by human peripheral blood mononuclear phagocytes. J Immunol 1991;146(1):63-7.

[258] Kagi D, Vignaux F, Ledermann B, Burki K, Depraetere V, Nagata S, et al. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. Science 1994;265(5171):528-30.

[259] Ennis FA, Cruz J, Spiropoulou CF, Waite D, Peters CJ, Nichol ST, et al. Hantavirus pulmonary syndrome: CD8+ and CD4+ cytotoxic T lymphocytes to epitopes on Sin Nombre virus nucleocapsid protein isolated during acute illness. Virology 1997;238(2):380-90.

[260] Huang C, Jin B, Wang M, Li E, Sun C. Hemorrhagic fever with renal syndrome: relationship between pathogenesis and cellular immunity. J Infect Dis 1994;169(4):868-70.

[261] Van Epps HL, Schmaljohn CS, Ennis FA. Human memory cytotoxic T-lymphocyte (CTL) responses to Hantaan virus infection: identification of virus-specific and cross-reactive CD8(+) CTL epitopes on nucleocapsid protein. J Virol 1999;73(7):5301-8.

[262] Mustonen J, Helin H, Pietila K, Brummer-Korvenkontio M, Hedman K, Vaheri A, et al. Renal biopsy findings and clinicopathologic correlations in nephropathia epidemica. Clin Nephrol 1994;41(3):121-6.

[263] Temonen M, Mustonen J, Helin H, Pasternack A, Vaheri A, Holthofer H. Cytokines, adhesion molecules, and cellular infiltration in nephropathia epidemica kidneys: an immunohistochemical study. Clin Immunol Immunopathol 1996;78(1):47-55.

[264] Mustonen J, Partanen J, Kanerva M, Pietila K, Vapalahti O, Pasternack A, et al. Association of HLA B27 with benign clinical course of nephropathia epidemica caused by Puumala hantavirus. Scand J Immunol 1998;47(3):277-9.

[265] Mustonen J, Partanen J, Kanerva M, Pietila K, Vapalahti O, Pasternack A, et al. Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. Kidney Int 1996;49(1):217-21.

[266] Vapalahti O, Lundkvist A, Vaheri A. Human immune response, host genetics, and severity of disease. Curr Top Microbiol Immunol 2001;256:153-69.

[267] Lucisano Valim YM, Lachmann PJ. The effect of antibody isotype and antigenic epitope density on the complement-fixing activity of immune complexes: a systematic

study using chimaeric anti-NIP antibodies with human Fc regions. Clin Exp Immunol 1991;84(1):1-8.

[268] Sansonno D, Dammacco F. Hepatitis C virus, cryoglobulinaemia, and vasculitis: immune complex relations. Lancet Infect Dis 2005;5(4):227-36.

[269] Lande MB, Mowry JA, Houghton DC, White CR, Jr., Borzy MS. Immune complex disease associated with Epstein-Barr virus infectious mononucleosis. Pediatr Nephrol 1998;12(8):651-3.

[270] Liu YF, Yang SJ, Yan PS, Liu YY. Characteristics of immunocomplex in autopsy tissues of hemorrhagic fever with renal syndrome. Chin Med J (Engl) 1994;107(6):444-9.

[271] Settergren B, Ahlm C, Alexeyev O, Billheden J, Stegmayr B. Pathogenetic and clinical aspects of the renal involvement in hemorrhagic fever with renal syndrome. Ren Fail 1997;19(1):1-14.

[272] Terajima M, Ennis FA. Quantitation of antibody-bound and unbound Sin Nombre virus in the plasma of patient with hantavirus pulmonary syndrome. J Virol Methods 2003;110(2):159-62.

[273] Paakkala A, Mustonen J, Viander M, Huhtala H, Pasternack A. Complement activation in nephropathia epidemica caused by Puumala hantavirus. Clin Nephrol 2000;53(6):424-31.

[274] Penttinen K, Lahdevirta J, Kekomaki R, Ziola B, Salmi A, Hautanen A, et al. Circulating immune complexes, immunoconglutinins, and rheumatoid factors in nephropathia epidemica. J Infect Dis 1981;143(1):15-21.

[275] Sulston JE, Horvitz HR. Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. Dev Biol 1977;56(1):110-56.

[276] Vaux DL, Weissman IL, Kim SK. Prevention of programmed cell death in Caenorhabditis elegans by human bcl-2. Science 1992;258(5090):1955-7.

[277] Feig C, Peter ME. How apoptosis got the immune system in shape. Eur J Immunol 2007;37 Suppl 1:S61-70.

[278] Cullen SP, Martin SJ. Mechanisms of granule-dependent killing. Cell Death Differ 2008;15(2):251-62.

[279] Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S, Sameshima M, et al. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 1991;66(2):233-43.

[280] Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995;3(6):673-82.

[281] Tartaglia LA, Rothe M, Hu YF, Goeddel DV. Tumor necrosis factor's cytotoxic activity is signaled by the p55 TNF receptor. Cell 1993;73(2):213-6.

[282] Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. Cell 1995;81(4):495-504.

[283] Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell 1995;81(4):505-12.

[284] Xu G, Shi Y. Apoptosis signaling pathways and lymphocyte homeostasis. Cell Res 2007;17(9):759-71.

[285] Lieberman J. The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal. Nat Rev Immunol 2003;3(5):361-70.

[286] Spaeny-Dekking EH, Hanna WL, Wolbink AM, Wever PC, Kummer AJ, Swaak AJ, et al. Extracellular granzymes A and B in humans: detection of native species during CTL responses in vitro and in vivo. J Immunol 1998;160(7):3610-6.

[287] Klingstrom J, Gudmundsdotter L, Zuber B, Hinkula J, Morner A, Wahren B, et al. Elevated levels of serum perforin in chronic HIV-1 and acute SIV/SHIV infection. Aids 2006;20(1):125-7.

[288] Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, et al. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 2002;111(3):331-42.

[289] Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 1998;94(4):491-501.

[290] Thomas DA, Du C, Xu M, Wang X, Ley TJ. DFF45/ICAD can be directly processed by granzyme B during the induction of apoptosis. Immunity 2000;12(6):621-32.

[291] Biven K, Erdal H, Hagg M, Ueno T, Zhou R, Lynch M, et al. A novel assay for discovery and characterization of pro-apoptotic drugs and for monitoring apoptosis in patient sera. Apoptosis 2003;8(3):263-8.

[292] Caulin C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. J Cell Biol 1997;138(6):1379-94.

[293] Linder S, Havelka AM, Ueno T, Shoshan MC. Determining tumor apoptosis and necrosis in patient serum using cytokeratin 18 as a biomarker. Cancer Lett 2004;214(1):1-9.

[294] Gold R, Schmied M, Rothe G, Zischler H, Breitschopf H, Wekerle H, et al. Detection of DNA fragmentation in apoptosis: application of in situ nick translation to cell culture systems and tissue sections. J Histochem Cytochem 1993;41(7):1023-30.

[295] Hay S, Kannourakis G. A time to kill: viral manipulation of the cell death program. J Gen Virol 2002;83(Pt 7):1547-64.

[296] Rao L, Modha D, White E. The E1B 19K protein associates with lamins in vivo and its proper localization is required for inhibition of apoptosis. Oncogene 1997;15(13):1587-97.

[297] Fanidi A, Hancock DC, Littlewood TD. Suppression of c-Myc-induced apoptosis by the Epstein-Barr virus gene product BHRF1. J Virol 1998;72(10):8392-5.

[298] Teodoro JG, Branton PE. Regulation of apoptosis by viral gene products. J Virol 1997;71(3):1739-46.

[299] Barry M, McFadden G. Apoptosis regulators from DNA viruses. Curr Opin Immunol 1998;10(4):422-30.

[300] Mi J, Li ZY, Ni S, Steinwaerder D, Lieber A. Induced apoptosis supports spread of adenovirus vectors in tumors. Hum Gene Ther 2001;12(10):1343-52.

[301] McCloskey TW, Ott M, Tribble E, Khan SA, Teichberg S, Paul MO, et al. Dual role of HIV Tat in regulation of apoptosis in T cells. J Immunol 1997;158(2):1014-9.

[302] Kang JI, Park SH, Lee PW, Ahn BY. Apoptosis is induced by hantaviruses in cultured cells. Virology 1999;264(1):99-105.

[303] Li XD, Makela TP, Guo D, Soliymani R, Koistinen V, Vapalahti O, et al. Hantavirus nucleocapsid protein interacts with the Fas-mediated apoptosis enhancer Daxx. J Gen Virol 2002;83(Pt 4):759-66.

[304] Michaelson JS. The Daxx enigma. Apoptosis 2000;5(3):217-20.

[305] Li XD, Kukkonen S, Vapalahti O, Plyusnin A, Lankinen H, Vaheri A. Tula hantavirus infection of Vero E6 cells induces apoptosis involving caspase 8 activation. J Gen Virol 2004;85(Pt 11):3261-8.

[306] Li XD, Lankinen H, Putkuri N, Vapalahti O, Vaheri A. Tula hantavirus triggers pro-apoptotic signals of ER stress in Vero E6 cells. Virology 2005;333(1):180-9.

[307] Markotic A, Hensley L, Geisbert T, Spik K, Schmaljohn C. Hantaviruses induce cytopathic effects and apoptosis in continuous human embryonic kidney cells. J Gen Virol 2003;84(Pt 8):2197-202.

[308] Brown M, Petric M. Evaluation of cell line 293 for virus isolation in routine viral diagnosis. J Clin Microbiol 1986;23(4):704-8.

[309] Khaiboullina SF, Rizvanov AA, Otteson E, Miyazato A, Maciejewski J, St Jeor S. Regulation of cellular gene expression in endothelial cells by sin nombre and prospect hill viruses. Viral Immunol 2004;17(2):234-51.

[310] Akhmatova NK, Yusupova RS, Khaiboullina SF, Sibiryak SV. Lymphocyte Apoptosis during Hemorragic Fever with Renal Syndrome. Russ J Immunol 2003;8(1):37-46.

[311] Li XD. Cellular mechanisms of hantavirus-induced apoptosis. Helsinki: University of Helsinki; 2005.

[312] Liu JM, Zhu Y, Xu ZW, Ouyang WM, Wang JP, Liu XS, et al. Dynamic changes of apoptosis-inducing ligands and Th1/Th2 like subpopulations in Hantaan virus-induced hemorrhagic fever with renal syndrome. Clin Immunol 2006;119(3):245-51.

[313] Enria D, Padula P, Segura EL, Pini N, Edelstein A, Posse CR, et al. Hantavirus pulmonary syndrome in Argentina. Possibility of person to person transmission. Medicina (B Aires) 1996;56(6):709-11.

[314] Lazaro ME, Cantoni GE, Calanni LM, Resa AJ, Herrero ER, Iacono MA, et al. Clusters of hantavirus infection, southern Argentina. Emerg Infect Dis 2007;13(1):104-10.

[315] 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.

[316] Toro J, Vega JD, Khan AS, Mills JN, Padula P, Terry W, et al. An outbreak of hantavirus pulmonary syndrome, Chile, 1997. Emerg Infect Dis 1998;4(4):687-94.

[317] Wells RM, Sosa Estani S, Yadon ZE, Enria D, Padula P, Pini N, et al. An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Hantavirus Pulmonary Syndrome Study Group for Patagonia. Emerg Infect Dis 1997;3(2):171-4.

[318] Martinez VP, Bellomo C, San Juan J, Pinna D, Forlenza R, Elder M, et al. Person-to-person transmission of Andes virus. Emerg Infect Dis 2005;11(12):1848-53.

[319] Wells RM, Estani SS, Yadon ZE, Enria D, Padula P, Pini N, et al. Seroprevalence of antibodies to hantavirus in health care workers and other residents of southern Argentina. Clin Infect Dis 1998;27(4):895-6.

[320] Navarette M, Pizarro E, Méndez C, Salazar P, Padula P, Zarov L, et al. Hantavirus ANDV distribution in human lungs and salivary glands. Abstract 103. In: VII international conference on HFRS, HCPS and hantaviruses; 2007; Buenos Aires, Argentina; 2007.

[321] Ferres M, Vial P, Marco C, Yanez L, Godoy P, Castillo C, et al. Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in chile. J Infect Dis 2007;195(11):1563-71.

[322] Vitek CR, Breiman RF, Ksiazek TG, Rollin PE, McLaughlin JC, Umland ET, et al. Evidence against person-to-person transmission of hantavirus to health care workers. Clin Infect Dis 1996;22(5):824-6.

[323] Wells RM, Young J, Williams RJ, Armstrong LR, Busico K, Khan AS, et al. Hantavirus transmission in the United States. Emerg Infect Dis 1997;3(3):361-5.

[324] Lee HW, van der Groen G. Hemorrhagic fever with renal syndrome. Prog Med Virol 1989;36:62-102.

[325] de Almeida Pdel V, Gregio AM, Machado MA, de Lima AA, Azevedo LR. Saliva composition and functions: a comprehensive review. J Contemp Dent Pract 2008;9(3):72-80.

[326] Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent 2001;85(2):162-9.

[327] Campo J, Perea MA, del Romero J, Cano J, Hernando V, Bascones A. Oral transmission of HIV, reality or fiction? An update. Oral Dis 2006;12(3):219-28.

[328] Murphy ME, Kariwa H, Mizutani T, Tanabe H, Yoshimatsu K, Arikawa J, et al. Characterization of in vitro and in vivo antiviral activity of lactoferrin and ribavirin upon hantavirus. J Vet Med Sci 2001;63(6):637-45.

[329] Murphy ME, Kariwa H, Mizutani T, Yoshimatsu K, Arikawa J, Takashima I. In vitro antiviral activity of lactoferrin and ribavirin upon hantavirus. Arch Virol 2000;145(8):1571-82.

[330] Valenti P, Antonini G. Lactoferrin: an important host defence against microbial and viral attack. Cell Mol Life Sci 2005;62(22):2576-87.

[331] Lee-Huang S, Huang PL, Sun Y, Huang PL, Kung HF, Blithe DL, et al. Lysozyme and RNases as anti-HIV components in beta-core preparations of human chorionic gonadotropin. Proc Natl Acad Sci U S A 1999;96(6):2678-81.

[332] Ferrari R, Callerio C, Podio G. Antiviral activity of lysozyme. Nature 1959;183(4660):548.

[333] McNeely TB, Dealy M, Dripps DJ, Orenstein JM, Eisenberg SP, Wahl SM. Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. J Clin Invest 1995;96(1):456-64.

[334] Ma G, Greenwell-Wild T, Lei K, Jin W, Swisher J, Hardegen N, et al. Secretory leukocyte protease inhibitor binds to annexin II, a cofactor for macrophage HIV-1 infection. J Exp Med 2004;200(10):1337-46.

[335] Bergey EJ, Cho MI, Blumberg BM, Hammarskjold ML, Rekosh D, Epstein LG, et al. Interaction of HIV-1 and human salivary mucins. J Acquir Immune Defic Syndr 1994;7(10):995-1002.

[336] Scott DA, Coulter WA, Lamey PJ. Oral shedding of herpes simplex virus type 1: a review. J Oral Pathol Med 1997;26(10):441-7.

[337] Slots J, Saygun I, Sabeti M, Kubar A. Epstein-Barr virus in oral diseases. J Periodontal Res 2006;41(4):235-44.

[338] Hermida M, Ferreiro MC, Barral S, Laredo R, Castro A, Diz Dios P. Detection of HCV RNA in saliva of patients with hepatitis C virus infection by using a highly sensitive test. J Virol Methods 2002;101(1-2):29-35.

[339] Abe K, Inchauspe G. Transmission of hepatitis C by saliva. Lancet 1991;337(8735):248.

[340] Ackerman Z, Ackerman E, Paltiel O. Intrafamilial transmission of hepatitis C virus: a systematic review. J Viral Hepat 2000;7(2):93-103.

[341] Mastromatteo AM, Rapaccini GL, Pompili M, Ursino S, Romano-Spica V, Gasbarrini G, et al. Hepatitis C virus infection: other biological fluids than blood may be responsible for intrafamilial spread. Hepatogastroenterology 2001;48(37):193-6.

[342] Marincovich B, Castilla J, del Romero J, Garcia S, Hernando V, Raposo M, et al. Absence of hepatitis C virus transmission in a prospective cohort of heterosexual serodiscordant couples. Sex Transm Infect 2003;79(2):160-2.

[343] Fabris P, Infantolino D, Biasin MR, Marchelle G, Venza E, Terribile Wiel Marin V, et al. High prevalence of HCV-RNA in the saliva cell fraction of patients with chronic hepatitis C but no evidence of HCV transmission among sexual partners. Infection 1999;27(2):86-91.

[344] Ferreiro MC, Dios PD, Scully C. Transmission of hepatitis C virus by saliva? Oral Dis 2005;11(4):230-5.

[345] Groopman JE, Salahuddin SZ, Sarngadharan MG, Markham PD, Gonda M, Sliski A, et al. HTLV-III in saliva of people with AIDS-related complex and healthy homosexual men at risk for AIDS. Science 1984;226(4673):447-9.

[346] Yeung SC, Kazazi F, Randle CG, Howard RC, Rizvi N, Downie JC, et al. Patients infected with human immunodeficiency virus type 1 have low levels of virus in saliva even in the presence of periodontal disease. J Infect Dis 1993;167(4):803-9.

[347] Rothstein TL, Mage M, Jones G, McHugh LL. Cytotoxic T lymphocyte sequential killing of immobilized allogeneic tumor target cells measured by time-lapse microcinematography. J Immunol 1978;121(5):1652-6.

[348] Kilpatrick ED, Terajima M, Koster FT, Catalina MD, Cruz J, Ennis FA. Role of specific CD8+ T cells in the severity of a fulminant zoonotic viral hemorrhagic fever, hantavirus pulmonary syndrome. J Immunol 2004;172(5):3297-304.