Diplomarbeit

Is Dobrava virus enzoonotic in Styrian yellow-necked mice?

eingereicht von

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Geb.Dat.: 25.12.1985

zur Erlangung des akademischen Grades

Doktorin der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an der

Sektion für Infektiologie und Tropenmedizin

der Universitätsklinik für Innere Medizin

unter der Anleitung von

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Graz, am 6.6.2014 Désirée De Simoni

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Acknowledgement

Hereby I would like to express my sincere gratitude to Univ. Prof. Dr. med. univ. Robert Krause for the facilitation of this thesis and his active support in the study regarding organization of location, permission of mousing, arranging of mouse examination as well as his vigorous support in catching and transportation of the animals, for which he sacrificed his precious time.

I would also like to manifest a special note of thanks to Univ. Prof. Dr. med. univ. Stephan Aberle and his team for the examination of mice and further diagnostics.

Abstract

Hantavirus diseases are emerging zoonoses with two manifestations of infection, Hantavirus Cardiopulmonary Syndrome in America and Hemorrhagic fever with renal syndrome in Eurasia. The case load is escalating worldwide and new viruses are found constantly in new territories. Transmission occurs via aerosolized excreta of carrier rodents. In Austria only Puumala and Tula virus were known to be endemic. In 2011, a case of presumably autochthonous acquired infection with a strain that was not known to circulate in the country before, was reported. This type, namely Dobrava-Belgrade virus, causes a more severe course of disease, with fatality rates of up to 12 %. Its reservoir, the yellow necked mouse, is widely distributed Austria. In order to prove the virus persistence in Styria, mousing was undertaken in the suspected area of virus acquisition and mice were screened for Hantavirus RNA via RT-PCR. No Dobrava-Belgrade virus was detected. However, physicians should be aware of this hazardous type which propagation in Austria could not be excluded.

The current thesis contains the actual knowledge about Hantaviruses in general, Dobrava-Belgrade virus in particular, the case report and our research project targeting the existence of Dobrava-Belgrade virus in local mice.

Preface

Hantavirusinfektionen zählen zu den neu aufkommenden Zoonosen und können zwei verschiedene Krankheitsbilder im Menschen auslösen. Die erste Form, Hantavirales Kardiopulmonales Syndrom (Hantavirus Cardiopulmonary Syndrome, HCPS), ist in Amerika verbreitet während das Härmorrhagische Fieber mit nephrotischem Syndrom (Haemorrhagic fever with renal syndrome, HFRS) in Asien und Europa vorkommt. Die Fallzahlen sind weltweit steigend und sowohl neue Viren als auch neue Verbreitungsgebiete werden regelmäßig identifiziert. Die Übertragung des Virus auf den Menschen findet vornehmlich über aerosolierte Partikel aus Mäuseexkrementen statt. In Österreich waren bisher nur Puumala- und Tula-viren, als krankheitsauslösende Hantavirussubtypen verbreitet, allerdings wurde 2011 von einem Fall einer vermutlich autochthon erworbenen Dobrava-Belgrad-virusinfektion berichtet, ein Stamm der davor nicht im Land zu zirkulieren schien. Genannter Typ verursacht einen meist schwereren Krankheitsverlauf, mit Fatalitätsraten von bis zu 12 %. Sein Wirtstier, die Gelbhalsmaus ist in Österreich weit verbreitet. Um die Präsenz des Dobrava-Virus in der Steiermark nachzuweisen, wurden Mäuse im vermeintlichen Ansteckungsgebiet gefangen und diese anschließend mittels RT-PCR auf Hantavirus-RNA untersucht. Obwohl in keiner der untersuchten Mäuse das Dobrava-Belgrad Virus gefunden werden konnte, darf die Verbreitung dieses gefährlichen Typs nicht als ausgeschlossen betrachtet werden, weshalb Ärzte in gegebenem Fall jedenfalls an eine solche Infektion denken sollten. Die vorliegende Diplomarbeit beinhaltet eine Zusammenfassung des aktuellen Wissens über Hantaviren im Allgemeinen, den Dobrava-Belgrad-Stamm im Speziellen sowie den Fallbericht und unser Projekt zur Untersuchung möglicher Existenz der genannten Hantavirusvariante in steirischen Mäusen.

Table of Contents

Glossary and list of abbreviations

RNA ribonucleic acid
NE: nephropathia epidemica
HPS: Hantavirus pulmonary syndrome
HCPS: Hantavirus cardiopulmonary syndrome
HFRS: hemorrhagic fever with renal syndrome
PUUV: Puumala virus
SAAV: Saarema virus
TULV: Tula virus
SEOV: Seoul virus
ICTV: International Committee on the Taxonomy of Viruses
ORNV: Oran virus
LANV: Laguna negra virus
LECV: Lechiguana
BMJV: Bermejo
ARAV: Araraquara
ANDV: Andes virus
BAYV: Bayou virus
BCCV: Black Creek Canal virus
NYV: New York virus
SNV: Sin nombre virus
CCHV: Crimean-Congo Haemorrhagic Fever virus
WNV: West West Nile virus
CHIKV: Chikungunya virus
SARS: Severe Acute Respiratory Syndrome
MERS: and Middle East Respiratory Syndrome
CoV: Coronavirus
MGLV: Monongahela virus
SANGV: Sangassou virus
MOUV: Mouyassué virus
AMRV: Amur virus
SOOV: Soochong virus
GOUV: Gou virus
SERV Serang virus
THAIV: Thailand virus
SEIR: susceptible (S), latent (E), infectious (I), recovered (R)
ETAR: 1-beta-d-ribofuranosyl-3-ethynyl-[1,2,4]triazole
FPI: N1-3-uorophenyl-inosine FPI
CRP: C-reactive protein
RT-PCR Reverse transcriptase polymerase chain reaction
MCHC: mean cell hemoglobin concentration
LDH: lactate dehydrogenase
APTT: activated partial thromboplastine time
CK: creatine kinase

Table of figures

List of Tables

List of Diagrams

1 Introduction and Background

Hantavirus, a genus of the Bunyaviridae family, represent a number of negative sense, single stranded RNA-viruses, some of which are pathogenic to humans and can cause two different diseases: Haemorrhagic fever with renal syndrome (HFRS), the milder form termed Nephropathia epidemica (NE), endemic in the Old World, and Hantavirus pulmonary syndrome (HPS), sometimes referred to as Hantavirus cardiopulmonary syndrome (HCPS), in the New World (1).

Hantaviruses are hosted by small mammals and unlike other Bunyaviruses, transmitted to humans mainly by aerosols originating from infectious excreta of the reservoir hosts (2), in rare cases also through bites (3) of the rodents who are chronically infected without presenting any symptoms of apparent disease (4).

Human to human transmission was reported only once for the Andes virus (ANDV) in America, but failing this, humans are dead end hosts for the species (5).

On average more than 200.000 Hantavirus cases are reported worldwide annually, with case severity ranging from sub-clinical to lethal (6).

In Europe, only five causative strains are endemic: Puumala virus (PUUV) prompting a mild form of HFRS (NE), Saarema virus (SAAV), which, like Tula virus (TULV), has so far only presumably caused a few cases of relatively mild HFRS, thus they are not unequivocally validated to be pathogenic, Seoul virus (SEOV) provoking a more severe form of HFRS (7), and Dobrava-Belgrade virus, triggering the most serious form of HFRS in Europe, with case fatality rates of up to 12% (8). Aggregated, they account for more than 10000 diagnosed (9) and 3000 hospitalized HFRS cases each year since 2000, which is almost double the incidence compared to the previous decades (10).

In addition to the quantity of cases, also the geographical radiation has magnified over the past years. The underlying principles for this remarkable virus spread and its future potential is subject to intensive research worldwide.

Since no existing vaccine or curative medicine for Hantavirus infections is available at the moment, it is crucial to monitor the emergence, geographical distribution, regional incidence as well as associated risk factors and the pathogenesis of the disease, for clinicians to suspect infections and diagnose infected individuals early on (11). The current knowledge about the virus is summarized hereinafter, with a focus on the Dobrava-Belgrade strain.

1.1 History

Hantavirus infections are often designated as emerging diseases, given the fact that increasing case numbers have occurred over the past years and ever more species and subspecies are identified and related to disease all over the world. However, the phylogeny of the main hosts and their viruses are remarkably concordant, revealing a long co-evolution over hundreds of years (6). Hjelle et. al. established the theory that Hantaviruses evolved in Eurasia and were propagated to the New World by rodents who crossed the Bering land bridge during Oligocene (33.9 million to 23 million years before present), and later spread to South America during Pliocene (5,332 million to 2,588 million years before present) (1).

It is unknown when the first human infection took place exactly, but there are indices, that Nephropathia epidemica-like diseases were acquainted in Asia for centuries, and could therefore be called a rediscovered disease (8, 12). It was also suggested, that not only “trench nephritis” during World War I could have been Hantavirus induced nephritis, but also the Leptospirosis-like outbreak in Finnish Lapland among German soldiers during World War II in 1942 (13). Other outbreaks of what is believed to have been HFRS were reported in Russia in 1913 and 1932, and among Japanese troops in Manchuria in 1932 (1).

Albeit confirmed records of a NE outbreak from Scandinavia of 1934 revealed the presence of the disease in Europe (1), it was not before a major outbreak of HFRS hitting US-army troops in the Korean war from 1951 to 1954, when more than 3000 soldiers fell ill, and 7% died (13), that the disease became known in the western hemisphere and was consecutively studied.

In 1978 Lee et al were able to identify an agent in the Korean striped field mouse, *Apodemus agrarius coreae* that was captured near the Hantaan river in Korea, as the causative virus for HFRS in humans (14), and therefore named it Hantaan virus (HTNV). In 1981, the HNTV was propagated in cell culture for the first time to be studied (15).

Thereat followed the detection of HNTV in *Apodemus agrarius coreae* and *Apodemus peninsulae* rodents in Far East Russia, China, South Korea and northern Europe (4). Dobrava virus (DOBV) and related strains in *Apodemus flavicollis*, *Apodemus agrarius* and *Apodemus ponticus* were found to host Hantavirus variants in many countries in Europe, see below (16).

Later, the rat-harbored Seoul virus (SEOV) was recognized to cause HFRS in Asia, and Puumala virus (PUUV), carried by the bank vole *Myodes glareolus* that was formerly known as *Clethrionomys glareolus*, to induce a milder disease in Europe (4). In Asia, *Clethrionomys* species were found to harbor PUUV related variants of Hantavirus (17).

Sin nombre virus (SNV) could be isolated from the deer mouse, *Peromyscus maniculatus*, within few weeks after the first outbreak of HPS occurring in the Four Corners Region of the US (New Mexico, Arizona, Colorado, Utah) in 1993. Since then, multiple virus strains causing HPCS were found throughout America, some of them causing case fatality rates of up to 40% (1).

The most important presently established American strains that can cause HCPS are: Sin Nombre virus (SNV), New York virus (NYV), Black Creek Canal virus (BCCV), Bayou virus (BAYV), Andes virus (ANDV), Araraquara (ARAV), Bermejo (BMJV), Choclo, Lechiguana (LECV), Laguna Negra (LANV), Oran (ORNV), Anajatuba, and Rio Maerim virus. The abundance of lately discovered Hantavirus isolates from shrews, moles and bats show no proven pathogenicity to this moment and will be briefly recited in chapter two.

Hitherto, 24 species are differentiated by the International Committee on the Taxonomy of Viruses (ICTV), many more are not yet classified and additional species or subspecies are identified continually.

Of the over 40 known species in 2014, 22 are designated pathogenic to humans, and all of the so far known unambiguously pathogenic Hantaviruses are rodent borne without exception (6).

1.1.1 Dobrava-Belgrade virus

As strain of interest in the underlying case report, the history of this type is described in more detail below.

The Dobrava-Belgrade virus species was first detected in an striped field mouse *Apodemus flavicollis*, near the Dobrava village in Slovenia in 1988, recognized as a new strain in 1992 (18), and following the isolation from a patient with HFRS in Belgrade 1992 (19), a comparison by nucleotide sequencing indicated, those two strains were identical (19) and therefore proposed and accepted to be named and officially listed as Dobrava-Belgrade virus (DOBV) in the ICTV since 1995.

This DOBV type from *Apodemus flavicollis*, then known as DOBV-Af, nowadays called Dobrava strain has successively been isolated in Greece 1996, Albania 1996, and Bosnia-Herzegovina 1997 (20). DOBV-neutralizing antibodies were found in patient sera from an outbreak of HFRS in 1991-92 in the Tula-Ryazan region, that were retrospectively studied in 1998, further in sera of two HFRS patients from Germany, and in Estonian patients 1998 (21). Serological studies have indicated the presence of DOBV-Af in Germany 1998, in Slovakia 1999, and the Czech Republic 2002 (20).

The detection of a Dobrava-virus from the rodent *Apodemus agrarius* succeeded in Estonia of the striped field mouse of Estonian islands Saarema and Vormsi 1999 (22). In Russia, Slovakia and Hungary this type was found in 1999, but has been then established as Saaremaa strain and not anymore classified as Dobrava subtype, albeit its close-relatedness (23).

Another distinct DOBV strain was recovered in the Kurkino region in Russia 1999 and in Slovakia 2001 in striped field mice (21) that is now reputed as Kurkino subtype. Later, DOBV was detected in Germany, Denmark and other parts of Russia where the correspondence of genome sequences from German and Russian patients could be proven.

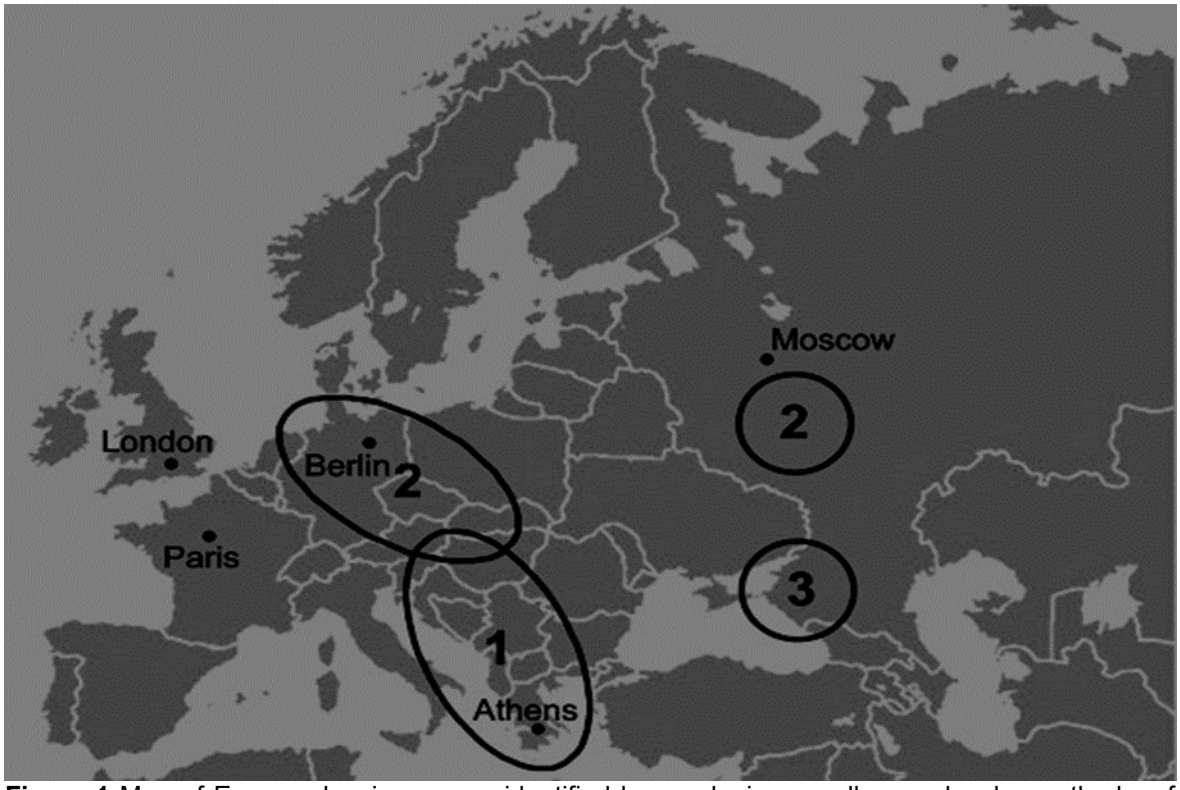

Figure:1 Map of Europe showing cases, identified by serologic as well as molecular methods, of hemorrhagic fever with renal syndrome caused by infection with the Dobrava-Belgrade virus (DOBV) variants: 1, DOBV-Af (Dobrava); 2, DOBV-Aa (Kurkino); and 3, DOBV-Ap (Sochi) (24)

The simultaneous existence of Dobrava subtype in yellow necked mice, the Kurkino variant and Saarema type in striped field mice as well as additional spillover occurrences complicated the matter and led to academic dispute. (24)
Supplementary, in *Apodemus ponticus*, a close-related species to *Apodemus flavicollis*, another DOBV strain was identified in the Black Sea region of Russia in 2006, intermittent named DOBV-Ap but now designated Sochi subtype (24).

1.2 Structure

The Bunyaviridae family comprises about 300 different enveloped, negative- or ambisense, single-stranded RNA viruses that infect animals, insects, and plants (25). Bunyaviruses form spherical or ovoid virions of 80 to 120 nm in size, sometimes elongated particles of 170 nm in length (13), which contain a linear RNA, tripartite into the small (S), medium (M) and large (L) segments.
Every segment is complexed with a protein, encoded by the S segment, that, indicating its function, is called the nucleocapsid protein (N), forming the three ribonucleic proteins. Those molecules present circular, because the 3´and 5´ termini of the linear segments include inverted complementary sequences that bond by base pairing (26).

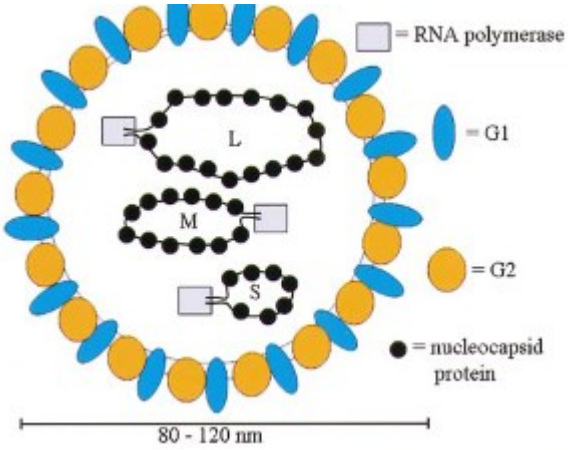

Figure 2: Schematic representation of a Hanta-virus particle. The letters L, M, and S identify the viral large, medium, and small single-stranded RNA genomes, respectively (13)

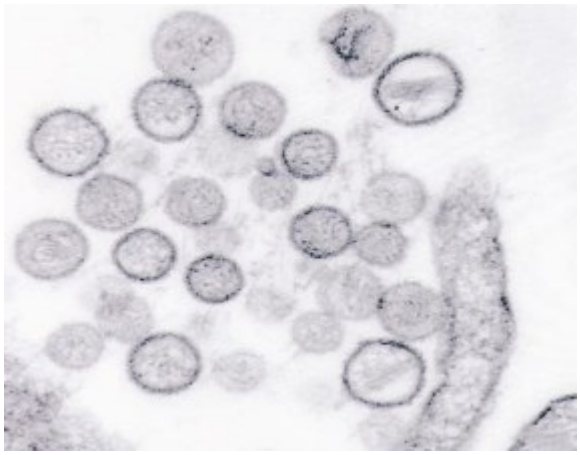

Figure 3: Thin section electron micrograph of Sin Nombre virus; magnification approximately ×45000. Photograph courtesy of the Centers for Disease Control and Prevention (13)

The N protein might also serve as a matrix protein, given the lack of such in Hantaviruses, presumably functioning as a mediator between the RNA molecules and the glycoproteins of the envelope (4). In some genera, a non-structural protein (NS) is also encoded by the S segment, for Hantaviruses this pertains to PUUV and TULV, (4) putatively also PHV, SNV, and ANDV (27), but not to the HNTV (28) or SEOV. This protein seems to interact with the IFN response of the infected cells (29). The glycoproteins, which are later integrated on the surface of the lipid bi-layer of the virion, and account for the grid like aspect typical for Hantaviruses (30), (4), originate from a precursor protein that is encoded by the M segment and co-translationally cleaved into the Gn and Gc proteins. The L molecule codes for the RNA-dependent RNA-polymerase (RdRp) that has also an endonuclease activity to split cellular mRNAs in order to initiate viral mRNA transcription by manufacturing capped primers in some members of the family (31). The RNA genome of Hantaviruses ranges between 11845 nucleotides for HTNV to 12317 nucleotides for SNV (4). Reassortments, scilicet exchange of segments can occur between different strains or even between different species when they infect the same host cell (32).

1.3 Epidemiology

A number of enzoonotic viruses have emerged throughout the world in the last decades, mostly by spilling over to new species or expanding their host range (33). Different potential causative factors are under suspicion of this phenomenon, including changes in human behavior leading to increasing frequency of human interactions with the carriers, nutrition-, education-, social-, and health-status, population development of the hosts, enhanced distribution, intensified pathogenicity due to viral evolution and climate changes (33). Improved recognition systems also count to potential causes that contribute to the general rise of reported incidents with new pathogens (4).

Along with Crimean-Congo Haemorrhagic Fever virus (CCHV), West West Nile virus (WNV), Chikungunya virus (CHIKV) (34), Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)-Coronavirus (CoV) (35) and others, Hantaviruses represent one of those pathogens, menacing humans to an ever-increasing extent. Hantavirus comprise an advancing global threat for public health, affecting approximately 150000 to 200000 humans worldwide

annually (36), with 90 % of the cases occurring in mainland China (37), but elevated case numbers throughout the globe. Moreover, the quantity of Hantavirus affected countries is constantly on the rise. The distribution of the virus is subject to intense research, the present knowledge of the underlying factors that drive the epidemics is outlined hereafter.

Hantaviruses have specific hosts, with each strain infecting only one, or closely related species of the same mammalian genus. In addition to this, the relations of the virus serotypes resemble the interrelations of the respective hosts, both facts seem indicative to a co-evolution of the virus with their primary reservoir; probably for thousands, or even millions of years (1), (6). The transmitting rodents don't show symptoms of infection, besides a slightly decelerated body growth (38, 39), and a suspected decreased life expectancy (40), underscoring the mutual interactions over the years, resulting in concurrent development (Figure 4).

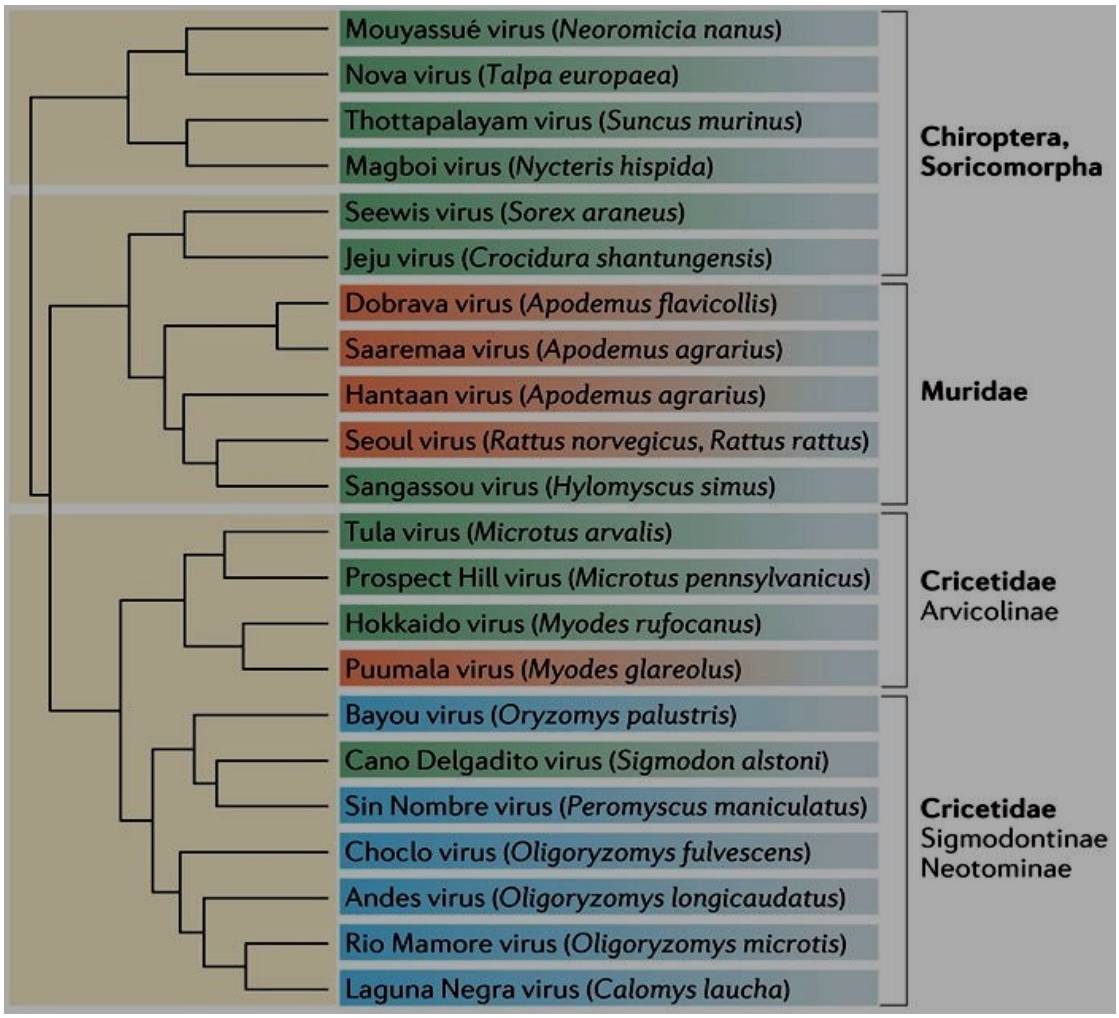

Figure 4: Relations of Hantaviruses and their respective primary hosts. HFRS-causing strains are red shaded (43).

However, new evidence contradicts this idea that Hantaviruses origin in rodents and co-evoluted with them. The presence of the virus in insectivores and bats hints to the potential existence of the species in other animal hosts (41). Those new detected strains do not form strict monophyletic groups, which indicates an evolution in different species as a result of cross-species host switching (42). Therefore, the strict co-divergence needs to be scrutinized in order to predict future evolutionary and biogeographic events more accurately (41).

Presently, each known pathogenic Hantavirus is exclusively transmitted by rodents of the *Muroidea* superfamily, within they can all be classified into *Muridae* family, comprising *Murinae* subfamily, and *Cricetidae* family, including *Arvicolinae*, *Sigmodontinae*, and *Neotominae* subfamilies (43).

The main representatives of each branch are Hantaan (HTNV), Seoul (SEOV), and DOBV, infecting the *Murinae* subfamily in Europe and Asia, Puumala (PUUV), the most abundant causative agent in Europe, infesting *Arvicolinae* species, Sin nombre (SNV) and Andes virus (ANDV), examples of American HCPS-evoking agents, transmitted by *Sigmodontinae* and *Neotominae* respectively (Figure 4) (24, 33, 44).

While the host-virus co-evolution theory appears to be true for the *Muridae*-born Hantaviruses, new research of *Chiroptera*- (bats) and *Soricomorpha*- (moles and shrews) born variants suggests that besides the described co-divergence, there seems to be evidence that cross-species transmission was crucial for Hantavirus evolution, and that in fact the virus primarily infested bats and insectivores (41). Thottapalayam virus (TPMV), detected in Asian shrews, is thought to have diverged early from rodent-borne Hantaviruses, supporting this hypothesis (45).

1.3.1 Host distribution

The universal existence of Hantavirus reservoirs throughout the globe has a tremendous disease-causing potential. However, because of the close-relatedness of the carrier-virus interaction, the geographic range of each Hantavirus is limited with few exceptions, while not necessarily determined by their natural host radiation (24). Therefore the classification of hosts and habitats of the reservoirs are of great interest.

1.3.1.1 America

In America a plethora of hosts and viruses are circulating. Of the *Neotominae* subfamily two species of the deer mouse, representing the most populous mammalian genus overall, namely *Peromyscus maniculatus*, and *Peromyscus leucopus,* are transmitter of Sin Nombre virus (SNV), New York virus (NYV) and Monongahela virus (MGLV) in the USA. Of the *Sigmodontinae* subfamily, that is endemic throughout the continent, two species are vectors of Hantaviruses in the US. The Black Creek Canal virus (BCCV), by *Sigmodon hispidus*, and the Bayou virus (BAYV) are transmitted by the marsh rice rat, *Oryzomys palustris*. The genus *Oligoryzomys*, also part of the *Sigmodontinae* subfamily, includes several species that are indigenous from Mexico to Tierra del Fuego (southern Chile) and endorse viruses to human in South America. MAPV is hosted by the delicate pygmy rice rat (*Oligoryzomys delicatus*). Another rice rate, *Oligoryzomys longicaudatus*, carries the hazardous Andes virus (ANDV) in Argentina and Uruguay, *Oligoryzomys chocoensis* the Bermejo virus (BMJV) in Bolivia, *Oligoryzomys fulvescens* the Choclo virus in Panama and *Oligoryzomys flavescens* the Lechiguana virus (LECV) in Argentina and Uruguay, *Oligoryzomys longicauda*tus the Oran virus (ORNV) in Argentina and *Oligoryzomys fornesi* the Anajatuba virus in Brazil. The Araraquara virus is transmitted by an unknown species in Brazil. The small vesper mouse *Calomys laucha,* also part of the *Sigmodontinae* subfamily, hosts the Laguna Negra virus in Bolivia and Paraguay, but is also endemic in Argentina, Brazil, Uruguay and Paraguay. Aun sin denominar virus is distributed by the closely related *Calomys laucha* in Paraguay and the Amazonian marsh rat *Holochilus sciureus* carries the Rio Maerim virus in Brazil, although it is native in all South America. Other apathogenic American strains and their carriers are not discussed in this context (2, 4, 43, 46, 47, 48).

1.3.1.2 Asia

In Asia, *Apodemus agrarius koreae*, carrier of HTNV and *Apodemus peninsulae*, host to the related Amur (AMRV) and Soochong (SOOV) viruses, are both endemic in the Far East, Russia, Korea and mainland China (4). Besides SEOV, which is carried by the universally endemic *Rattus norvegicus*, as mentioned above, the

related Gou virus (GOUV) is also carried by rat species, namely the *Rattus rattus*. The Asian house rat, *Rattus tanezumi*, inhabiting Southeast Asia, distributes the Serang virus (SERV). The greater bandicoot rat *Bandicota indica* and the lesser bandicoot rat *Bandicota savilei* both carry Thailand virus (THAIV) (4). Four novel apathogenic Hantaviruses, Huangpi virus, Lianghe virus, Longquan virus, and Yakeshi virus, were recently detected in China and seem to be distinct from known Hantaviruses. Huangpi virus was found in *Pipistrellus abramus* (Japanese house bat), Lianghe virus in *Anourosorex squamipes* (Chinese short tailed shrew, familiy *Soricidae)*, Longquan virus in *Rhinolophus affinis, Rhinolophus sinicus*, and *Rhinolophus monoceros,* (horseshoe bats) and Yakeshi virus in *Sorex isodon* (Taiga shrew, family *Soricida*e), respectively (41). Hantavirus RNA was also detected in Pomona roundleaf bats *Hipposideros pomona* (roundleaf bats), captured in Vietnam in 1997 and 1999 (49). The Thottapalayam virus, hosted by *Suncus murinus* (Asian house shrew) was recently found in China, Nepal, India and Vietnam (41).

1.3.1.3 Africa

In Africa, the Sangassou virus (SANGV), one of the closest relatives of DOBV was found in Guinea 2006 in the African wood mouse (*Hylomyscus simus*), a species that is distributed all over sub-saharian Africa (50). Another strain, hosted by the banana pipistrelle (*Neoromicia nanus*), which is endemic all over the continent, was captured near Mouyassué village in Côte d'Ivoire in June 2011 and therefore named Mouyassué virus (MOUV) (51). Two African Hantaviruses were detected in shrews that are inhabiting the savanna in western Africa, namely Azagny virus, harbored by the West African pygmy shrew (*Crocidura obscurior*) in Cote d'Ivoire and Tanganya virus, found in Therese's shrew (*Crocidura theresae*) in Guinea. Another bat-borne strain was isolated from the hairy slit faced bat (*Nycteris hispida*), also endemic in west-african savanna. The said specimen was captured near the Magboi River in Sierra Leone (52, 53).

1.3.1.4 Europe

In Europe, the vector of the PUUV, the bank vole*, Myodes glareolus*, is endemic west of the Ural Mountains throughout the continent, except in Mediterranean coastal regions, the Iberian Peninsula and Greece (8). Their haunt consists of forests and forest edges, a characteristic it shares with *Apodemus flavicolli*s, which

carries the DOBV Af strain, now named Dobrava, as well as spillover strains from DOBV Ap variant, now established as Kurkino subtype. The so called yellow necked mouse is inhabiting woods all over Europe, excluding the western coastal regions, Northern Scandinavia and the British Isles (4). The distribution of the striped field mouse, *Apodemus agrarius* overlaps with the habitat of the yellow necked mouse, but is mainly residing east of the former iron curtain and not spread in Western Europe and, other than the first, prefers cultivated areas, city parks and field edges (8). In addition to the low pathogenic SAAV, the stripe field mouse carries the Kurkino virus and spillover strains of DOBV Af. *Apodemus ponticus*, carrying the Sochi virus, formerly known as DOBV-Ap, propagates this virus in Caucasian and Transcaucasian regions (54). Many other wood mice, *Apodemus*, are also disseminated throughout Europe including Mediterranean islands and Northern Africa, but were not yet identified as Hantavirus reservoirs while bearing a great potential of hosts.

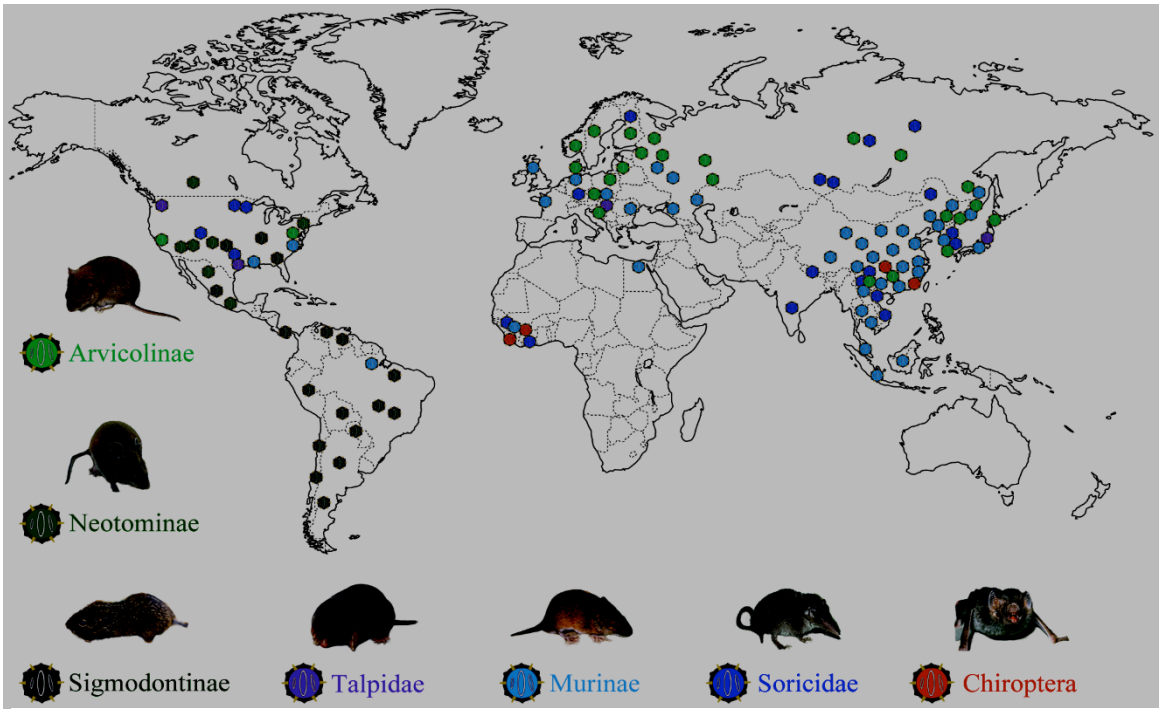

Figure 5: A map of the world illustrating the location of known Hantaviruses by host group and associated mammalian hosts. **(**41)

SEOV is carried by *Rattus norvegicus, Rattus losea* and *Rattus rattus,* which are distributed all over the world. TULV that has been linked to human infection but not definite to disease, is spread in the common vole, *Microtus arvalis*, that dwells central and eastern Europe, several other *Microtus* species and the

water vole *Arvicola amphibius* (22). Topograf virus has only been detected in Siberia where the siberian lemming *Lemmus sibiricus* is endemic (8). Laihia, Asikkala and Seewis virus are examples of apathogenic viruses, found in Finland and Germany, and are carried by the Eurasian water shrew, *Neomys fodiens*, the Eurasian pygmy shrew, *Sorex minutus* and the common shrew *Sorex araneus*, respectively (55).

1.3.1.4.1 Austria

The main case load occurs in Styria, the south eastern part of Austria bordering Slovenia, and Carinthia near to Styria, where many different types are indigenous. However, so far only PUUV and TULV has been verified to circulate in their corresponding hosts *Myodes glareolus*, and *Microtus arvalis*, with confirmed spillover to humans and consecutive disease (55). One case of DOBV infection, that was presumably acquired autochthonous, has been reported so far (56). Two primal host species of the detected strain are endemic throughout the country, namely the yellow-necked mouse (*Apodemus flavicollis*) and striped field mouse (*Apodemus agrarius*). Although no illnesses have been linked to other potential Hantavirus carriers, includeing other Apodemus species, representatives of the *Rattus* subfamily, some additional *Microtus* variants as well as a selection of shrews and moles, they are endemic in the area (48).

1.3.2 Transmission

Humans are spillover hosts, acquiring infections primarily through the inhalation of aerosolized excreta from the infected mammals. There are also some indications of transmission through bites. However, while this route seems to have a great importance for virus circulation amongst reservoir individuals, it was only very rarely reported in human infections (3).

The transmission between humans could so far only be shown in ANDV. Although this way of virus dissemination would imply a great hazard, it has fortunately been proven that person-to-person transmission of the virus depends on the extent of intimate contact with infected individuals. The risk of transmission was 17.6% among sex partners and 1.2% among household contacts (4, 11, 57).

RNA of PUUV was found in saliva of patients, but factors within the fluid appear to possess inhibitory quality, for which ANDV seems to be less vulnerable than other Hantaviruses and can therefore possibly be transmitted through the liquid (4, 58).
Ingestion of contaminated food was hypothesized to play a role in transmission as successfully demonstrated in Syrian hamsters by intragastric administration of virus, but has not been proven to have major epidemiological influence (59, 60).
Vertical transmission was reported to happen in HFRS patients (61), but those findings could not be replicated in recent studies with DOBV and PUUV infected pregnant women. In those studies, transmission to the child could not be verified for HFRS (62). There was also no evidence for vertical transmission of HCPS caused by SNV (63). Nowadays Hantaviruses are not considered to be transmissible to the child during pregnancy (2).
Investigations yielded, that HFRS infection was acquired at increased frequency during outdoor activities such as farm work, threshing, camping, military exercises, and other rural and forest related activities. Indoor contact with the virus occurred primarily via exposure to contaminated dust, preferably in the course of cleaning basements, sheds, cabins or any other closed, non-aerated, unused buildings that were priory infested with rodents (1, 11, 64). PUUV for example remains contagious at room temperature in excreta for two weeks, in absence of UV-light and in colder temperatures probably even longer (4, 65).
High prevalence of disease was also linked to recreational activities (66), and it was shown that 97% of DOBV-associated HFRS infections happen in rural environments, where *Apodemus* species breed in preference (24).
In general the de-urbanization, that was intensified in the past two decades and claimed to improve health, has perhaps also made humans more susceptible for zoonotic pathogens through increased contact with wildlife (10).
While rodents represent the reservoir of pathogenic Hantaviruses besides shrews moles and bats, some other domestic and wild animals like cats, dogs, pigs, cattle, and deer showed seropositivity against the agent in question. Domestic animals live in a similar habitat as infected primary hosts, suggesting that these become infected from contact with the rodents. This would be possible, if the target organs, tissues, and cell parenchyma of spillover animals had adequate receptors and were suitable for virus entry and replication (33). Each cross-species transfer, likely resulting in change of ecology and environment, bears a great potential of virus adaption that

could lead to a reinforced pathogenicity and virulence of the virus. Therefore prevention of such events could be advanced by regularly screening domestic animals in high endemic areas (6, 33).

1.3.3 Factors that drive epidemics

A lot of research has been done in the last decade, to shed light on the growing incidence of Hantavirus infections around the globe, in order to predict impending future epidemics and to take precautionary measures to prevent major outbreaks. It is not yet clarified entirely how the dissemination is driven, but several factors are under suspicion to impact the propagation and transmission of the virus (67). For transmissible diseases in general, as early as 1966, the Russian epidemiologist Pavlovsky determined five requirements on which the virus circulation in specific geographic landscapes depends. Those are animal donors, vectors, animal recipients, the pathogenic agent itself in an infective state, the influence of factors of the external environments contributing to an unhindered spread of pathogenic agent (68). Therefore, factors that interfere in those preconditions represent relevant variables in the dissemination of the zoonosis.

For Hantavirus in particular, components that potentially interfere, were identified as follows: first, factors that can evoke changes in the density and diversity of host population, like climate changes, landscapes, changes in land-use, biodiversity, food availability and physiological features of reservoir population; second, variables that are dependent on virus features, their evolution and their interactions with infested individuals, among them prevalence of infection in the reservoir host, viral excretion in the environment, survival of the virus outside its host; third, factors that interfere with the main spill-over route to humans through inhalation of infected rodent excreta including behavioral factors of human and rodents. Of course some of these factors interact and can be affected themselves by other factors. It appears, that the subject is complex and follows diverging laws in varying geographical areas (67).

Rodent density appears to play a prominent role in transmission rate to humans. The oscillations in hospitalization frequency could to some extent be linked to variations in host population. This construct was intensively studied for PUUV, and the HFRS case load was proven to be related to the abundance of *Myodes glareolus.* The underlying reasons for the numerical enlargement of the species

differ geographically (4, 67, 69). In most of Europe, peaks in quantity of mice are preceded by high summer temperatures two years in advance, and high autumn temperatures one year prior, both conditions leading to a masting phenomenon, that provides an opulence in food availability (70). In different European regions, the diet of both *Apodemus flavicollis* and *Myodes glareolus* differs as well. In Western Europe, mainly oak and beech seed crops are used for energy intake, while in central Europe, oak and hornbeam crops are regulators of population size (55). *Apodemus agrarius* is less dependent on forestry impacts but more to anthropogenic factors, because it prefers a mixed habitat of field and forest (55).

In northern Europe on the contrary, only small number of predators as result of harsh winters, are able to keep the rodent growth at bay, therefore oscillations in predator density is reversely proportional to prey count, resulting in regular cycles of *Myodes glareolus* abundance. In northern Sweden and Finland, a two year time lag between population crash and peak could be observed (71). The triggering of mast years is not the only relevant result of climatic phenomena. From 2006 to 2007, a sudden warm period in the midst of a normal winter pushed the bank voles closer to human settlements, leading to a large Hantavirus outbreak in Sweden. In China, low rainfall, combined with the existence of good crops lead to an abundance of *Apodemus agrarius,* that in turn lead to higher case numbers of HFRS (4).

Landscape composition constitutes the next variable in reservoir and virus propagation. Not only the existence of burrow space, but also moderate rainfall, semi-humid soil type, mild air and soil temperature, little elevation and agricultural land use are significant for inhabitation (72). Deforestation, presence of overstorey and denser vegetation were also identified to conduce population growth (4).

Whereas abundance of reservoir species is an important element in Hantavirus epidemics, and obviously a certain threshold needs to be exceeded, it is not the only determining aspect of virus diffusion. The geographic range of the disseminating species is not always congruent with the complete distribution area of the same species. This constraint to a confined number of infested animals is not understood to date (43).

A high biodiversity of animals is generally held accountable for a pathogen dilution effect, that means, through high species diversity of both hosts and non-carrying animals, the prevalence is diminished because host encounters, altered host behavior, host survival, and therefore host density is also decreased. Hence, a

decreased biodiversity, occurring due to intensive agriculture, pesticide use, insecticide use urbanization and deteriorating landscape could contribute to the Hantavirus accumulation. However, the topic is controversial. Antithetic to the dilution effect, an amplification effect was postulated, that is, greater species diversity leads to more frequent encounters between hosts or via secondary hosts resulting in higher prevalence of virus.

This points once again to the complexity of the relationships between abiotic and biotic parameters, rodents, humans, and Hantavirus infections (67). Perhaps newly invented epidemiological calculation programs could help to shed more light on the matter and render epidemic predictions possible (4).

Those mathematical models try to describe dynamics of host-virus interaction of zoonotic viruses; how they are maintained in their reservoirs; the processes that lead to transmission, host switching, recombination, re-assortment, and molecular events that result in the transfer and adaptation to a new species. Deterministic models differentiate rodents as either susceptible (S), latent (E), infectious (I) (viral shedding), or recovered (R) (no viral shedding) (SEIR model) (73, 74).

For Hantavirus, the models enter differences in male and female seroprevalence, density-dependent survival, lack of vertical transmission and random mating into the equation. If environmental stochasticity is included, those simulations show that Hantavirus outbreaks can be triggered by a vast enlargement of rodent population numbers, but they also show, that due to stochastic variability, this is not always the case, as said a certain threshold of density seems to be required for the happening. Consequences of spill-over incidents are also taken into account, given the potential contribution of those events to virus maintenance and evolution.

Mathematical models can lead to the discovery of new ecological paradigms and a true understanding of the nature of episodic zoonotic epidemics such as those caused by Ebola virus, SARS CoV, Nipah virus, and Machupo virus as well as many new viruses, including Hantaviruses, that remain to be discovered (4).

1.3.4 Prevention of infection

Considering the absence of curative treatment and protective immunity in the human population as well as the vast potential of virus dispersal, preventive

measures are the most effective method to contain the spread of Hantavirus infections at the moment. Different efforts have so far proven to be protective.

Exposition prophylaxis: Prophylaxis of spillover is best accomplished by avoidance of interaction with rodents and their excreta. In endemic areas it is therefore recommended to wear face masks and disposable gloves while being in rooms with potential mice infestation. Dust swirling should especially be avoided in such locations as stables, sheds, summer houses after winter and basements etc. (60). Since people who have recreational or occupational contact to contaminated rodents, for example campers, hikers, farm workers and woodcutters, are at higher risk for infection, they should particularly take measures to avoid contact to rodents and their excreta (75). As part of that, the safe storage of food, disinfection and disposal of trapped or dead mice, reduction of shelter opportunities to prevent mice from entering dwellings as well as elimination of the rodent should be undertaken. The disinfection of contaminated areas can be accomplished by steeping surfaces with 10% household bleach solution (13). In order to avoid transmission under laboratory conditions, rodents held for research should be screened for Hantavirus infections (60).

Host-reservoir control and human exposure prophylaxis interventions, have led to a dramatic reduction of human cases in China over the past decades (11).

HCPS-causing Hantaviruses are Bio-defense Category A pathogens as classified by the anti-bioterrorism program of the US National Institutes of Allergy and Infectious Diseases. As a consequence of the consecutive research funding for prevention and therapy of the syndrome, and the installation of large programs to enhance awareness of the disease, to accelerate diagnoses and improve intervention measures with prompt transportation to centers with intensive care units and aggressive management of the complications, great improvements could be noted in several countries. In Chile, as most outstanding example, the fatality index has been lowered from 60% to around 30% only due to better management and prevention measures (6).

Another strategy for reducing incidence of Hantavirus infection besides minimizing the risk of exposure could be the effective strategic vaccination of populations in areas of endemicity or of risk groups in military, clinical, and research settings (4).

Vaccination

Several vaccines for HFRS are in use for humans in Asia. Vaccines based on inactivated virus were approved for human use in 1993. Those substances, propagated in different cell lines or mouse brains were targeted either solely against HTNV or additionally to PUUV or SEOV as bivalent agents.

Hantavax is a monovalent inactivated vaccine against HTNV and distributed in Korea. In China, bivalent vaccine for HTNV and SEOV, cultivated in Vero cells (an African green monkey kidney cell line), is in use since 2003. Since 2008, Hantavirus vaccine is included in the national Expanded Program on Immunization in China and free of charge for people living in highly endemic areas for HFRS. About two million people are vaccinated each year with only minor side effects. Although the vaccination is described as safe and efficacious, these qualities are questioned in other studies. At the moment no vaccine is approved in neither Europe nor America. Extensive research regarding Hantavirus vaccines is undertaken around the globe. Table 1 shows a summary of the most developed vaccines to date.

Summary of hantavirus vaccines used in humans and non-human primates

Hantavirus	Vaccine format	Immunogen(s)	State of development	Country
HTNV	Inactivated virus	Whole particle	Human mass vaccination	South Korea
HTNV, PUUV	Inactivated virus	Whole particle	Clinical studies	South Korea
HTNV, SEOV	Inactivated virus	Whole particle	Human mass vaccination	China
HTNV	Recombinant vaccinia virus	G_N, G_C, N	Clinical studies (not pursued)	US
HTNV, PUUV	DNA vaccine	G_N, G_C	Clinical studies	US
ANDV, HTNV, PUUV, SEOV	DNA vaccine	G_N, G_C	Pre-clinical studies (Rhesus, Cynomolgus)	US

Table 1 Summary of Hantavirus vaccines used in humans and non-human primates (60)

Putative vaccine approaches in vaccination development include reassortants between pathogenic and apathogenic live viruses, molecular chimeric viruses, virus like particles, recombinant proteins and DNA vaccines. Their value remains to be determined in the future (4), (60), (76).

Passive immunization approaches and chemo-prophylaxis were proposed for prevention of infection of persons at risk, like household contacts of ANDV infected patients, or people with high occupational risk for infection. Those methods are outlined in chapter 1.7.

1.4 Clinical features

Depending on the virus species and subspecies, the clinical appearance ranges from mild to lethal. While the identification and categorization of the viruses can facilitate a better assessment of the expected severity of the illness, every individual infection is different and can be potentially life threatening. The case fatality rates for DOBV, HTNV, SEOV and PUUV induced HFRS are estimated 12%, 5%, 2%, and 0.08% respectively (6).

1.4.1 HFRS

The clinical course also differs between the various strains and individuals, but commonest proceeds as follows: After the incubation period, which averagely lasts two to four weeks but can range within the scope of 10 days to 6 weeks (44), HFRS presents in five successive, sometimes overlapping stages: the febrile, the hypotensive, the oliguric, the diuretic and the convalescence phase. Not all of them must be distinguishable or even present in every patient (8). The disease usually starts with unspecific, influenza like symptoms, high fever up to four days, headache, myalgia, arthralgia, nausea, vomiting, diarrhea and abdominal pain (55), as part of the febrile phase that lasts three to seven days in most cases. The gastrointestinal symptoms can present as acute abdomen leading to misdiagnoses, and sometimes surgery (6). Rarely, also neurological symptoms such as retrograde amnesia, cognitive deficit and paresis of legs, and cardiovascular symptoms show up during this prodromal phase (77), (78). Somnolence and visual disturbances are frequently reported. Lens thickening, leading to a myopic shift and blurred vision is pathognomonic of HFRS, (8) but its prevalence appear to diverge geographically and is not correlated to the severity of the case (79).

In a study, performed by Hautala et.al. over 80% of the patients experienced each decreased intraocular pressure, reduced visual acuity, conjunctival chemosis and

thickening of the lens. Myopic shift, shortening of the anterior chamber and reduction of vitreous body length were also diagnosed in more than half of the cases. In total 70% of the patients reported ocular disturbances (80). In another report from Slovenia, about 80% of PUUV-infected patients and 40% of DOBV-infected patients are afflicted with blurred vision during acute HFRS. Photophobia has been reported regularly (81), (82). Sometimes hemorrhage generates mucosal and conjunctival injections or flushing of the face during the febrile phase. A petechial rash occasionally appears, preferably on axillary folds and palate. Severe HFRS manifests with severe albuminuria that often commences suddenly on the fourth day and is an omen for a severe course (1, 13).

The sequent hypotensive stage, enduring about two to seven days, is often characterized by nausea and vomiting and accompanied by thrombocytopenia and sometimes mental confusion (6). Vascular leakage and cardiogenic shock, happening in almost 15% of the patients during this period (13), account for one third of all Hantavirus-related deaths (1).

During the ensuing oliguric period, lasting about five days, patients are at risk for hypertension, pulmonary edema (6), and commonly develop renal impairment or failure, resulting in the requirement of hemodialysis. Lung or kidney failure and cardiogenic shock due to hypovolemia can also bring about lethal outcome at this stadium, during which half of all fatalities occur (1).

The diuretic phase, originating in the lack of urine concentration capability of the kidneys last several weeks to months and generally ends in full recovery in the majority of cases (60), but a vulnerability to shock or pulmonary complications remains existent. About half of HFRS patients are found to have abnormalities in the electrocardiogram. The final convalescent phase may last months before curing is complete (1, 4).

Typical abnormal laboratory findings encompass thrombocytopenia, leukocytosis, accelerated erythrocytes, elevated serum creatinine and urea, hemoconcentration, higher levels of transaminases, hematuria, proteinuria, and high C-reactive protein levels; elevated levels of serum lipase and amylase as signs of acute pancreatitis were detected in several cases. Cases of multiorgan failure have been reported (24, 83, 84, 85).

Some rare manifestations and complications in association with HFRS are ARDS, arthritis, polyradiculitis, seizures, urinary bladder paralysis, encephalitis, Guillain-

Barré syndrome, acute disseminated encephalomyelitis, perimyocarditis, pulmonary infiltrates or edema, hemorrhage and necrosis of pituitary gland and hemorrhages spleen and other organs (8, 86, 87, 88, 89, 90).
There are indications of late sequelae after HFRS due to hanta-viral infection. Mesangiocapillary glomerulonephritis, panhypopituitarism, hypertensive renal disease and mild tubular lesions with hypertension are found amongst those long lasting complications (8, 91, 92).

1.4.1.1 Infections with Dobrava-Belgrade virus

The infection with this subtype usually shows a severe course of HFRS as described above, with differences between the single strains. Dobrava has a mortality rate of 12%, Sochi shows fatalities in 6% and Kurkino has a lethal outcome in only 0.3 - 0.9% of cases (21). The symptoms are generally more severe: haemorrhagic complications, petechiae, rash, and mucosal injection, disseminated, intravascular coagulopathy, thrombocytopenia, hypotension, hypertension, shock, oliguric renal failure requiring dialysis treatment, retroorbital edema, panhypopituitarism, pulmonary edema, pleural and abdominal effusions, pancreatitis, gastrointestinal and electrocardiography disorders are more frequently encountered, more evident and severe (8, 83, 93, 94, 95). Neurological symptoms as coma, impaired consciousness, delirium or peripheral facial nerve palsy are relatively common, but brain magnetic resonance imaging or electroencephalogram findings are seldomly abnormal (82, 83, 96).
Typical laboratory findings in Dobrava infections are found in high percentages of cases: proteinuria (100%), increased serum creatinine (95–100%), thrombocytopenia (50–75%), leucocytosis (50%), microscopic haematuria (58–85%), increased C-reactive protein (up to 96%), raised serum transaminases (41–68%) and electrolyte imbalance (8).

1.4.2 HCPS

This disease has high fatality rates up to 40% but is not endemic in Europe. However, besides the scientific significance due to the commonalities it shares with

HFRS, it is of clinical importance for physicians to recognize this hazardous infection in imported cases. Symptoms vary, but a common feature of both HFRS and HCPS is increased permeability of the vessels and mononuclear infiltration (97). Table 2 summarizes the different manifestations of HCPS and HFRS.

Disease	Pathogens	Distinguishing Characteristics*
HFRS (moderate-severe) Death rate 1%-12%	HTNV, SEOV, DOBV	hemorrhage +++
		proteinuria +++/++++
		pulmonary capillary leak +/++
		myositis +/+++
		conjunctival injection ++/++++
		eye pain/myopia ++/++++
HFRS (mild) Death rate <1%	PUUV	hemorrhage +
		proteinuria +/++++
		pulmonary capillary leak -/+
		myositis +
		conjunctival injection +
		eye pain/myopia ++/++++
HCPS (prototype) Death rate up to 40%	SNV, NYV	hemorrhage +
		proteinuria +
		pulmonary capillary leak ++++
		myositis -
		conjunctival injection -/+
		eye pain/myopia -
HCPS (with renal manifestations) Death rate up to 40%	BAYV, BCCV, ANDV	hemorrhage +
		proteinuria ++/+++
		pulmonary capillary leak +++/++++
		myositis ++/++++
		conjunctival injection -/++
		eye pain/myopia -

Table 2: Clinical manifestations of HFRS and HCPS; Minimum/maximum occurrence of the characteristic: - rarely reported; + infrequent or mild manifestation; ++, +++, ++++ more frequent and severe manifestation (1).

HCPS has an incubation period of seven to 39 days. Usually the syndrome takes a course through several stages, starting with prodromal symptoms that resemble HFRS to a great extent and last five days at the maximum. They can include fever, myalgia, weakness, headache, back pain, abdominal pain, nausea, vomiting and diarrhea. Patients typically appear at the clinic with cough, dyspnoea, fever,

tachycardia, headache and hypotension, symptoms that usually unfold on second to fourth day of disease and are signs of an already advanced HCPS. Because of its fulminant course, rapid diagnosis is essential. Most deaths occur in the first 48 hours after onset of symptoms due to cardiogenic shock and respiratory failure originating in rapidly progressive pulmonary edema caused by capillary leakage and low cardiac output. Renal failure with anuria, myositis, mild hemorrhagic disturbances such as hematuria, hematemesis, intestinal bleeding, and metrorrhagia were observed. Elevation of pancreatic enzymes and encephalitis has also been reported in HCPS (98, 99, 100).

Laboratory findings include signs of metabolic acidosis, lymphocytopenia, leukocytosis with left shift, hyponatraemia, elevated creatinine and urea. High hematocrit and progressively decreasing platelet levels are strong indicators for HCPS. Blood O2 saturation often falls below 90%, increased blood levels of aspartate transaminase (AST) and alanine aminotransferase (ALT) are found regularly. IgM and IgG antibodies appear shortly after the onset of the prodrome. Bilateral diffuse lung interstitial infiltrates that evolve to alveolar infiltrates are observed in chest radiographies in the majority of the cases and rales are often heard in physical examination (101, 102).

Respiratory failure and consecutive shock often require mechanical ventilation, vasoactive drug support or extracorporeal membrane oxygenation. Both resolve mostly within a few days and are sometimes followed by a polyuric phase. The renal affection, a result of prolonged hypo perfusion, varies amongst the different New World Viruses. Renal impairment and chemical evidence of skeletal muscle inflammation are rarely observed in SNV. In contrast ANDV, BAY, and BCC viruses are associated with renal insufficiency and elevated CK levels at much higher frequency although also not universally (1, 99). Patients may suffer from symptoms of fatigue, myalgia and dyspnea for months; there are reports that in numerous cases, sequelae remained for up to 2 years after acute infection (6, 100).

Severity and case-fatality rates of HCPS vary geographically. Acute infection by most New World hantaviruses, including Sin Nombre, Andes, Araraquuara and Juquitiba viruses, show case-fatality rates of 25% to 40%, whereas the Choclo virus in Panama and LNV in Paraguay, areas of high seroprevalence, the mortality rates is as low as 10% and 15% respectively (6).

1.5 Pathogenesis/Immunology

1.5.1 Host infection

Hantaviruses produce a chronic infection with no apparent harm in their natural hosts (24). Similar to several emerging enzoonotic viruses, the persistent infection is characterized by the absence of conspicuous pathology (103), except for a slightly retarded body growth and a reduced lifespan of the carrier (38, 39, 104).

The immunological response of the rodent reservoir is complex and only starting to be uncovered (97). The transfer among the reservoir species occurs mainly through biting and inhaling of aerosols, while the offspring seems to be protected by maternal antibodies for some time (47). Vertical transmission could only be demonstrated for BCCV in cotton rats (*Sigmodon hispidus*) (31).

Within a few days after infection, immune genes are expressed as a result of immune activation. This stimulation of the innate and adaptive immune system shows to be less intense than that in pathology models (47). Despite evidence of immune responses, the virus happens to remain persistent in the hosts, which seem to shed it over long periods. Indeed infectious virus was found in their lungs from day 14 to day 270 after inoculation and feces and urine appeared to contain contagious particles 35 to 130 days post inoculation of rodents (103).

In concordance with human infection, endothelial cells in different organs represent the main target (44). While virus replication takes place in those tissues, it doesn't lead to vascular leaks in affected host animals. This circumstance could be explained by a different immune response pattern. Inoculation of *Rattus norvegicus* with SEOV and deer mice with SNV respectively, revealed a dominant regulatory T-cell response (105). Those regulatory T-cells are capable of suppressing pro-inflammatory and effector T cell activity (Figure 6).The T-cell responses from the *Peromyscus maniculatus* showed, after infection with SNV, expression of several

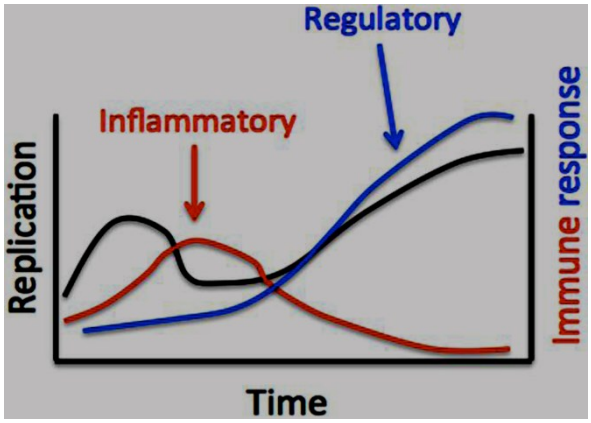

Figure 6: Model of the immune response of deer mice infected with SNV. During acute infection, SNV elicits a modest inflammatory response that initially limits, but does not clear, virus. Within a few weeks, the response transitions to a regulatory response that may allow episodic recrudescence of virus that can be shed (47).

cytokines, amongst them IFN-γ, IL-4, IL-5, and TGF-β1, but not TNF, lymph toxin, or IL-17. All lines of regulatory T cells from inoculated deer mice synthesized TGF-β1, which seems to hold immunopathology in the host at bay (106). Alveolar macrophages and lung micro-vascular endothelial cells of rats, productively infected with SEOV, did also not induce pro-inflammatory responses in either tissues, instead TGF-β1 expression was induced while other T-cell related markers were not. The cells allowed virus replication in absence of cytokine or chemokine induction, supporting the theory that Hantavirus is able to avoid antiviral response in host species (107). SEOV RNA was also found to be reduced in lungs and saliva parallel to reduction of regulatory T-cell activation in Norwegian rats. The evaluation of lung pathology yielded multifocal hemorrhage and edema after inactivation of regulatory T-cells. That confirms the theory that regulatory T cells promote anti-inflammatory immune response while preventing the virus from being eliminated through elevated transcription and synthesis of TGF-β and suppression of TNF (108). This response may prevent viral clearance, an assumption that was proven to be true in other viral diseases. Such a response might have originated as a bilateral beneficial concept between Hantaviruses and their rodent reservoirs, limiting pathogenicity while allowing the virus to remain in a population (47). Another striking feature of Hantavirus infection in rodents is a difference between immune responses of male and female individuals. A number of immune-related genes were expressed at altering amounts. Female specimens had a significantly higher quantum of transcription factor, T-cell, pro-inflammatory, antiviral T cell and Ig family member genes, presumably diminishing their importance as participants in the transmission cycle. It is yet unclear why this is the case, and along with many other features of mammalian infection needs to be researched further (105). This difference could not be demonstrated unambiguously in human infection. Gender related differences in pathogenicity and immunity, observed by manifestation of symptoms, clinical parameters and mortality were identified amongst human infected individuals with HFRS in Sweden, Finland and China, but could not be verified in Germany (109).

1.5.2 Human infection

The immunological mechanisms behind Hantavirus infections are very complicated and, given the lack of adequate animal models, not yet entirely encoded. Two New World Hantaviruses ANDV, and non-pathogenic Maporal virus MAPV are found to cause HCPS-like disease in Syrian golden hamsters, *Mesocricetus auratus* (47). Unfortunately, those models are not applicable for HFRS. However, the crab eating macaque *Macaca fascicularis*, (also called cynomolgus monkey), showed some HFRS symptoms after inoculation with PUUV, allowing some insight to immunology. *Macaca fascicularis* produced elevated levels of nitric oxide, various cytokines IL-10, IL-6, and TNF-α, and C-reactive protein after inoculation with PUUV (110). Other research was mostly accomplished under laboratory conditions.

The absence of cyto-pathogenicity against infected cells in vitro indicates a major role of the human immune system as factor that appears to trigger, or at least enhance, the destructive course of the infection, although the humoral system seems to provide a protective function. The exact role of the immune system remains to be revealed in detail (60).

The main element in the pathogenesis of both HFRS and HPS is increased vascular permeability in the primarily targeted endothelial cells of lung and kidney capillaries, but also of other organs. The specific ability of the virus to favor one tissue over the other is not understood completely, anyway the distinction is not sharp in all cases. Both manifestations of Hantavirus infection are associated with acute thrombocytopenia, changes in vascular permeability and both can present with renal and pulmonary symptoms (60). Furthermore, epithelial cells, macrophages, follicular dendritic cells, lymphocytes, neutrophils and platelets are infested in the two diseases (8).

After the inhalation of the virions, macrophages and dendritic cells might serve the virus to spread throughout the body. The first are suggested to serve as vehicle for dissemination, because of their existence in the primary contacted lung tissue as alveolar macrophages, the latter, because of their consecutive migration to lymph nodes (60). Hantaan virus was substantiated to infect dendritic cells and induce their maturation. This activation lead also to up-regulation of major histocompatibility complex class I (MHC-I) and intracellular adhesion molecule 1 (ICAM-1) on the cell surface. By release of tumor necrosis factor (TNF) alpha and beta, both pro-

inflammatory cytokines, and activation of T-cells, dendritic cells apparently do not only contribute to the virus distribution but also to the pathogenesis (111).

The mechanisms of the consecutively induced vascular leak is also not entirely understood to date, but various mechanisms appear contemplable, including sensitizing to vascular endothelial growth factor (VEGF), down-regulating of vascular endothelial cadherin, induction of natural killer cells by increasing MHC molecules and ICAM, equal to the mechanism in dendritic cells, or triggering CD8 positive T-cells to target infested endothel which appears to be the prime mechanism (107). While apathogenic viruses are thought to use the beta1-integrin receptors for the cell entry process, pathogenic strains require beta3-integrin as well as decay accelerating factor (DAF) and glycoprotein p33, alternately (112). After successful infection, IgM and IgA antibodies rise very quickly, IgG and IgE responses set in deferred. The role of IgE molecules, regarding virus clearance and pathogenesis was not identified so far, although they were detected to be increased in HFRS patients (60). The antibodies are mainly targeted against the N protein. Reactions with Gc and Gn protein occur delayed. It is an unsettled matter why N protein dominates in induction of immune cells over Gc and Gn antigens, although the surface antigens seem to be the easier accessible targets (113). It has been suggested that the N protein epitopes represent the best conserved and most abundant expressed antigen during infection, but a viral distraction method has also been discussed (60). Either way, the immunity against reinfection lasts presumably for life. Neutralizing antibodies were detected years after SNV, ANDV, (114) and PUUV infection respectively. Given the rise of neutralizing antibodies long time after infection of individuals who were provably not exposed again, lead to speculations about viral persistence (115).

Cross neutralization of distinct Hantavirus types with diverging success could be demonstrated in a number of studies, which indicates that the B-cell epitopes of Gn, Gc and N-proteins are conserved in to varying degrees amongst different Hantavirus strains (60). Virus reactive T-cells are in an analogous manner predominantly targeted against N-protein epitopes, while Gn and Gc proteins play a tangential role as antigens (60). Because Hantavirus, as has been proven, is non-cytopathic in infected cells in vivo and in vitro, it has been hypothesized, that the immune response is mainly accountable for the pathogenesis (6), particularly cytotoxic CD8 positive T-cells; however the subject is controversial. Supporting this notion, in

patients with severe acute HCPS, higher frequencies of specific T-cells were detected compared to SNV-infected individuals showing less severe symptoms (60). On the other hand, a higher quantity of T-cells during HFRS is associated with a milder manifestation of disease (116). One explanation for these diverging results might be the difference in methods to measure T-cells, missing the distinctions between T-cells. They can in fact be divided into cytolytic and non-cytolytic cells. The non-cytolytic types were shown to secrete IFNγ, and help to control non cytopathic viruses. In accordance with this, IFN producing T-cells are likely to support the clearance of Hantaviruses. The cytolytic T-cells, however, might contribute to a large extent to the vascular leakage (60).

Importantly, there is an association between T-cell response and mild disease with better outcome lying on the basis of genetic predisposition of HLA type. HLA-A*2 and HLA-B*35–positive patients had a better outcome of HFRS caused by HTNV, and the latter were shown be of advantage in ANDV infection (4, 117). The role of VEGF and VEGF-sensitizing of endothelial cells could not be substantiated yet, and it seems that VEGF is more important for repairing mechanisms after infection (78). The speculation about increased degradation of the adherence junction protein, vascular endothelial (VE)-cadherin, due to Hantavirus infection as causative mechanism of vascular leakage could not be verified. Pathogenic Hantavirus infections did not cause any degradation of VE-cadherin in vitro experiments (118). However, reduced VE-cadherin expression on the surface of ANDV-infected endothelial cells raises doubts on the definiteness of the matter (119). Another mentioned Hantavirus-related mechanism disease suggested to interfere with the virulence was IFN. Pathogenic viruses are under suspicion to regulate the early IFN induction to replicate successfully. Their replication was blocked by IFNα/β, as long as it was added prior, or within 12 hours of infection. Later addition had scarcely any effect on virus replication (120).

The second ostensible characteristic of Hantavirus infection, the evident thrombocytopenia, is far from being clarified. Theories regarding this peculiarity include platelet consumption in response to the damage to the endothelial layer and direct consequence of the virus interaction with beta-3-integrin receptor on platelets (6). In summary, it can be stated that the fascinating immunopathogenesis of Hantavirus remains enigmatic to a great extent to date and bears a vastness of potential future approaches for therapy and vaccination.

1.6 Diagnosis

Upon the onset of symptoms of vascular leakage, virtually in all acute HFRS and HPS cases, antibodies to viral proteins are detectable. All three proteins (Gn, Gc, and N) can induce a high level of IgM at the onset of symptoms, but the IgG response to the glycoproteins may be delayed, and in the prodromal phase, there remains a possibility that both markers are below detectable rate. Also the responses against glycoproteins Gn and Gc are not of great diagnostic importance, because they arise later in the course of infection and are much less conserved among different strains. Therefore, serological tests that determine IgM antibodies to hanta-viral N-protein in serum are the most common approaches for the diagnosis of HCPS and HFRS (4, 6, 13, 121, 122, 123).

1.6.1 Serological diagnostics

One of the first tests for diagnoses of HFRS was the indirect immunofluorescence assay (IFA) in which Hantavirus-infected cells were fixed on microscope slides. The use of virus-infected cells is now obsolete, antigens are mostly N proteins derived by different recombinant expression systems that were found to be functioning for the matter. Because the N protein epitopes are widely conserved among hanta-viral strains, there exists a high cross-reactivity between closely related Hantaviruses, especially within each phylogenetic group (*Murinae*-borne, *Arvicolinae*-borne, *Sigmodontinae*-borne and *Neotominae*-borne). Therefore differentiation between them is not always possible. If this conservation is not apparent, another problem arises for tests using only a single antigen. Given the fact that Hantavirus serotypes co-circulate in parts of Europe (PUUV, DOBV and SAAV), Russia (SAAV, PUUV, DOBV and HTNV) and Asia (HTNV, SEOV and PUUV), monospecific tests may fail to detect the strain entirely. Nevertheless, IFAs using one or two serotypes of Hantavirus are widely used as diagnostic tool (4, 13, 124, 125). The N protein is also used in immunoenzymatic assays (EIA) as well as in strip immunoblot tests, both instruments for detecting antibody reaction. Nowadays, the most common tests for detection of Hantavirus infection represent indirect IgG and IgM enzyme-linked immunosorbent assays (ELISA) and IgM capture ELISAs. The rapid IgM capture ELISA is effective for the diagnosis of HFRS and HPS within minutes. An immunochromatographic test for the fast approval of HFRS and HPS has been

tested. Assays for simultaneous detection of antibodies against Old and New World Hantaviruses have also been developed to advance diagnosis, which is crucial in light of the unspecific prodromal symptoms and the potentially fulminant course (6, 121, 122, 124, 126, 127, 128). Neutralization tests are rarely in use for diagnosis and typing of suspected infections, because they are laborious and require BSL-3 laboratories. However, they allow distinction between related infections serologically. Focus reduction neutralization tests could be used as a routine test for the determination of serological hanta-viral infections. Plaque reduction neutralization test (PRNT) and Cross-PRNT can be utilized to distinguish and also measure antibody levels but were shown to be less specific for human acute-phase sera (4, 13, 114, 129).

1.6.2 Molecular diagnostics

The reverse transcriptase–polymerase chain-reaction (RT-PCR) as a highly sensitive diagnostic test based on the detection of the virus genome proves useful for both HFRS and HPS and the resulting data may be of epidemiological value. It offers a direct detection of the viral genome hence allowing the identification of the underlying hanta-viral genotype, for this purpose it is superior to serological tests. On the other hand, RT-PCR technology is more complex and its availability limited. Due to the hazardous nature of hantaviruses in aerosols that can form while handling the agent, the serological or molecular tests of supposedly contaminated tissues and serum require BSL-3-rated or BSL-4 laboratories.

The Hantavirus genome can then rapidly be detected within blood, serum, or organ fragments, from the first day after the onset of illness. For HCPS, RT-PCR was shown to be successful during the first 10 days of illness and viral genomes were even detected before the first day of symptoms. Testing of whole blood samples appeared to be advantageous to serum testing. Quantitative PCR on whole blood samples of household contacts of ANDV index cases turned positive up to 5 to 15 days before serological diagnosis and onset of HCPS.

In general, primary amplification of hanta-viral RNA from cell culture can be performed by RT-PCR. However, the low levels of viral RNA present in human and rodent tissue samples can require nested-RT-PCR techniques using primers

selected for regions with high homology. Nested-RT-PCR tests have been developed, for HTNV, SNV, PUUV and ARAV.
Virus isolation must also be performed in BSL-3 or BSL-4 laboratories, but it is difficult, usually inefficient and therefore not routinely performed. Primary isolation of Hantaviruses is mostly undertaken by the use of Vero cells in which the virus usually does not develop cytopathic effects. The virus isolation is then accomplished by isolating RNA from the infected cells after about 2 weeks of incubation and performance of RT-PCR. A useful diagnostic tool in future attempts to isolate Hantavirus from humans might be utilization of an immunofluorescence assay to screen seropositive patients serum for virus production in VERO E6 cells before confirmative PCR (4, 6, 13, 57, 130, 131, 132).

1.7 Treatment

To this date there is no causative curative treatment available. Several approaches were made to find an effective antiviral drug and the research is ongoing. At the moment, the only agent with some efficacy against Hantavirus replication is Ribavirin. It showed to reduce mortality and to improve the outcome in animal trials. In HFRS patients it proved a seven-fold reduction in morbidity and a significant reduction in fatalities. Another study from Korea showed a lower rate of oliguria and no need of dialysis treatment in the cohort treated with Ribavirin. Altogether a decreased severity of renal insufficiency was evident. However, those effects seem only to be present if the drug is administered very early in the course and there are controversies about side effects and benefits of the drug. It appears to be very unspecific in terms of virus type and results of HCPS patients receiving Ribavirin were inconclusive. Probably the efficacy depends on the severity and phase of disease on first admission of Ribavirin (60), 133, 134, 135). Studies are still ongoing to clarify the role of Ribavirin in HFRS (e.g. 144). In HPS Ribavirin has been shown to have no effect (145, 146).
Other promising antiviral agents with some efficacy against Hantaviruses are 1-beta-d-ribofuranosyl-3-ethynyl-[1,2,4]triazole (ETAR), with a similar protective virtue as Ribavirin, N1-aryl purines as a structural class with the representative N1-3-uorophenyl-inosine (FPI) proving some activity against HTNV, and the group of substances that target the αvβ3 receptor by binding the β3 integrin to block virus

entry and replication as multivalent cyclic peptides presented on nanoparticles, or peptidomimetic compounds that showed potency against Hantavirus entry to a 2,000 times fold higher extent than the cyclopeptide. However, the therapeutic potential of these compounds as well as their pharmacokinetic needs to be further investigated in living models (60, 102, 136, 137).

Passive immunization

Since analyses of patients showed an apparent viremia during acute phase of HFRS, and high antibody titers are often correlated with a rather benign clinical course of infection, the post infection prophylaxis with antibodies appears promising. Hitherto no controlled clinical trial has been carried out for passive immunization of HFRS or HCPS patients. However, both polyclonal and monoclonal antibodies were demonstrated to be effective in animal models (60, 138, 139, 140).

HTNV Gc-specific neutralizing monoclonal antibodies, administered up to 4 days after challenge with virus cured infected hamsters. Primates were protected from PUUV by passive immunization with neutralizing antibodies to the virus. Passive transfer of sera from rhesus macaques vaccinated with the M segment of ANDV was shown to protect against lethal infection. Those findings suggest that even if complete inhibition of virus replication is not evident, patients could benefit from prophylaxis treatment regimen if sufficient antibody concentrations are present during the acute infection. This treatment regimen was effective in other viral diseases such as, hepatitis A and B, and Varicella viruses so the future outlook for this type of therapy is auspicious (60, 138, 139, 140).

Conservative treatment

Facing the absence of any causative cure to HFRS, the treatment is basically supportive. Maintaining the fluid balance and circulatory volume is crucial, albeit dangerous overhydration for patients that are anuric and with leaky capillaries must be avoided. It depends on the fluid status, diuresis and kidney function how much liquid needs to be replaced and it is to be monitored carefully. Patients with renal insufficiency and or pulmonary edema may need dialysis treatment. Mechanical ventilation could be essential in case of lung involvement. NSAIDs should be omitted in pharmacotherapy for headache, but paracetamol can be used and is needed in many cases. Thrombocytopenia and severe hemorrhage can lead to the necessity

of a transfusion, and prolonged low platelet counts were reported to be successfully treated with prednisolone. The treatment can encompass further measures depending on the course and complications. Treatment of shock, neurological manifestations hypopituitarism, pancreatitis and many other manifestations can be of the essence and potentially demand special care but will not be discussed in this context.

HCPS treatment calls for prompt action. The treatment is also primarily conservative targeted on the abrupt respiratory failure and shock. In general the therapy is recommended to be handled in cardiopulmonary intensive care units where blood and tissue oxygenation, cardiac output, central blood pressure and cerebral pressure can be measured. Facing pulmonary edema and hypotension, maintaining oxygenation is essential. Intubation and mechanical ventilation is usually required, extra-corporal membrane oxygenation might become vital. Possible complications and implications might also demand special care depending on the varying degree of severity and manifestation (8, 36, 141, 142, 143).

2. Material and Methods

For the theoretical part of the Diploma thesis, literature review was undertaken during 2013 and 2014. In the process, pubmed was searched and studies were reviewed, including results until April 2014. The focus was set on current relevant studies regarding the subject, mainly discovered by keyword search of Hantavirus Epidemiology, Phylogeny, Immunology, Transmission, Prevention and Dobrava-Belgrade virus. In addition to studies that were made available to me by Univ. Prof. Dr. med. univ. Krause, mainly free full text articles and articles available by the Medical University Library of Graz were used as basis for information acquisition.

For description of the case data were retrieved from medical records of the patient and electronic databases.

For investigation of suspected Dobrava virus infestation of local mice population mice were captured. During May and June 2012, mice were caught around the supposed area of Dobrava virus acquisition in St. Radegund near Graz 47° 10′ 52″ N, 15° 29′ 22″ E and surrounding forests by the author of this thesis (DdS) and the supervisor (RK). The investigation has been approved by the Amt der

Steiermärkischen Landesregierung, Fachabteilung 8C - Veterinärwesen, Styrian Government.

In four different sites of the region, deathtraps were set and bated with chocolate cream. The first locus was the empty attic of a former farm building, the second a shack and its close surroundings in the woods, the third a cellar of a restaurant and the fourth the area around a close by field. Traps were screened for captured mice on a daily basis. The caught mice were documented by photography, labelled and gathered in a freezer of the Medical University of Graz. Mice were classified by the phenotype on the pictures with support of a specialist in the field. Subsequently the frozen mice were transported to the laboratory of Univ. Prof. Dr. Aberle at the Department of Virology, Medical University of Vienna, where they were dissected and again classified by molecular methods. The organs of each mouse were tested for Dobrava-Belgrade and Puumala virus by use of RT-PCR.

3. Case Report

Anamnesis

Patient X, a 54 year old man was admitted to the emergency unit, Department of Internal Medicine, Medical University of Graz, by the emergency service which he called himself after experiencing flickering in his eyes and pre-collapsing state on 25th of August 2011. He appeared in reduced health condition, and reported unbloody diarrhea, malaise and nausea for three days, but no vomiting. Subjectively he was very sick and weak. The patient stated that there was no recent stay abroad and no ill persons he was aware of having contact with.

Medical findings on admission:

General state: decreased, dietary condition: normal, heart rate: 84/min; spO2 96%, temperature 37,7°C, blood pressure 125/80 mmHg; no cyanosis, no signs of anemia, no icterus; head/neck: normal; pharynx bland, jugular veins not engorged, no meningism; auscultation of heart and lungs: normal, abdomen: abdominal wall below chest level, no tenderness of palpation, no resistance, peristaltic sounds normal in all four quadrants, renal bed: no pain on palpation, basic neurological examination normal, extremities: no evidence of deep venous thrombosis (DVT),

No edemas; digital rectal examination: no stool, no blood; hemoccult test non possum, no stool;

Electrocardiographic findings: sinus rhythm 80/min, intermediate heart, PQ 0,14s; R/S transition in V3/V4, intermittent negative T in III, incomplete right bundle branch block (RBBB);

Arterial blood gas analysis:

	standard value	25.08.11
Temp a	°C	37
Baro a	mmHg	730
pO2	71-104 mmHg	99,7
phosphate	7,370-7,450	7,51
pCO2	35-46	15,7
akt HCO3	21-26	12,5
BE	-2 to 3mmol/l	-7
BEecf a	-2 to 3mmol/l	-10,2
sO2 a	95-98,5 %	98
hb total	13-17,5 g/dl	18,7
O2Hb a	%	96,8
COHb a	%	0,5
MetHb a	%	0,7

Table 3: Arterial blood gas analysis on admission

Laboratory findings:

Elevated erythrocytes, hemoglobin, hematocrit, mean cell hemoglobin concentration (MCHC), thrombopenia, lymphopenia, hyponatreamia, hypochloremia, decreased GFR, elevated creatinine, urea, transaminases, LDH, APTT and CRP (Table 4).

Assessment/implemented therapies:

Patient was put under observation in the emergency unit and received ELO-MEL isoton 1500 ml, one vial of Paspertin and Pantoloc 40 were administered intravenously. Another blood test to monitor kidney parameters and hemogram was ordered for 6pm. The results showed a slight decline of creatinine to 1, 37 mg/dl (standard value 0, 70-1, 20 mg/dl) there was no significant change in blood count and electrolyte balance.

	standard value	25.08.2011 15:07
haematology		
leucocytes	4.4-11-3 G/l	5,72
erythrocytes	4.5-5.9 T/l	6,5
haemoglobin	13-17.5 g/dl	20,5
haematocrit	40-50%	52,8
MCV	80-98 fl	81,2
MCH	28-33 pg	31,5
MCHC	33-36 g/dl	38,8
thrombocytes	140-440 G/l	61
differential blood count, mech.		
neutrophil granulocytes	50-75 %	76
neutrophil granulocytes abs.	1.8-7.7 G/l	4,4
eosinophil granulocytes	-5%	0
eosinophil granulocytes abs.	-0.7 G/l	0
basophilic granulocytes	-1%	1
basophilic granulocytes abs.	-0.2 G/l	0,1
monocytes	2-12 %	7
monocytes abs.	0.2-1.0G/l	0,4
lypmphocytes	20 40%	16
lypmphocytes abs.	1.0-4.8 G/l	0,9
electrolytes		
sodium	135-145 mmol/l	130
potassium	3.5-5.0 mmol/l	4,5
chloride	95-110 mmol/l	90
Ca 2+ frdP	1.15-1.35 mmol/l	1,1
Ca2+ges	2.2-2.65 mmol/l	2,33
kidney		
creatinine	0.7-1.2 mg/dl	1,95
urea	10-45 mg/dl	60
GFR a. MDRD	80-140 ml/min	35,94
liver		
GGT 37°C	- 55 U/l	42
AST 37°C	-35 U/l	104
ALT 37°C	-45 U/l	71
heart		
CK 37°C	-170 U/l	137
LDH 37°C	120-240 U/l	496
carbohydrate metabolism		
glucose	70-115 mg/dl	149
lactate	0,5-2,2 mmol/l	2,4
coagulation		
PT	70-120%	86
PT-INR	INR	1,1
APTT	26-36 sec	42,1
fibrinogen	210-400 mg/dl	327

Table 4: Laboratory analysis on admission

Further proceedings: At 9 pm the patient still suffered from diarrhea and was at his own request administered to a private clinic (Krankenhaus der Elisabethinen Graz) on 25th of August 2011.

Because of acute renal failure and proteinuria he was transferred back to the University Hospital on 27th of August, where he was admitted to the Department of Nephrology.

Clinical course:

The medical findings of the physical examination resembled those established on first admission, only the laboratory findings were indicative for renal failure, furthermore a thrombopenia, leucocytosis and elevation of CRP were present Subsequent ultrasound examination of the kidneys was unconspicuous. On 28.8.2011 the laboratory findings revealed a creatinine of 5, 76 mg/dl, urea of 161 mg/dl, uric acid of 8, 8 mg/dl and GFR of 10, 30 ml/min. Further thrombopenia, leucocytosis 21, 08 G/l, lymphopenia 40 G/l, hematocrit of 38, 7%, MCHC of 38, 2 g/dl and elevation of CRP, biochemical pancreatitis were present. The urine test showed a severe proteinuria of 400 mg/dl, a specific weight of 1,013, and 330 erythrocytes/microl. The 24h urine collection revealed a creatinine of 0,1g (standard value 0, 8-2, 5 g/24h), urea of 0, 8 g (standard value 10-25 g/24h), protein concentration of 14314 mg/l (standard value -130 mg/l) and a total protein excretion of 2862, 80 mg (standard value -110 mg/24h). The ongoing aggravation of laboratory parameters and general state towards oligoanuric renal failure, with profuse formation of edema lead to the conduction of several diagnostic tests on 29th of August. Stoll culture did not show Salmonella, shigella, yersinia, campylobacter, noro-virus-RNA, leukocytes or clostridium difficile and C. diff. toxins. The quick test for IgM against leptospirosis was negative, but the Hantavirus test showed positive IgM against PUUV and HTNV, which lead to the request of an infectious disease service consultation for advice on further diagnostic and therapeutic steps. In detailed history the patient denied any stay abroad stays or cleaning attics, huts or basements. He lived in a rural area, where cats prevail and kill mice regularly. He recently cut the grass during dry periods where he could be exposed to aerosolized mice excreta. In addition, he was used to run daily to an adjacent hill where he had direct contact to forest soil possibly contaminated by mice excreta. A subjective improvement of general condition could be noticed. He did not have blurred vision, but the sense of a retro orbital pressure. In accordance to the

consulting Infectious disease specialist serum and EDTA blood was sent to the Department of Virology, Vienna, for further Hantavirus examination including PCR and serological confirmation of the in house test. Further the patient was seen in the Department of Ophthalmology in order to assess the depth of the anterior chamber. The ophthalmic examination revealed conjunctival involvement, but the depth of the anterior chamber was within normal ranges, therefore involvement of eyes was excluded. Therapy was initiated with Oculosept and Bepanthen eye ointment. The Infectiouse disease specialist further recommended instant performance of blood gas analysis in case of dyspnea, thoracic CT and in the event of HCPS, preterm admission to ICU. Additional tests that were routinely performed revealed low vitamin D3 of 23,3 ng/ml (standard value 30-60 ng/ml) but normal calcitriol of 45 pmol/l, negative HBVs antigens, HBVc antibodies, positive HBVs, and HAV IgG antibodies, consistent with immunization status, and negative HIV tests. Autoimmune antibodies were inconspicuous and the iron metabolism was slightly imbalanced with values for Fe of 39 µg/dl (standard value 50-160 µ/dl), transferrin of 1520 g/l (standard value 2000-3600 g/l) and ferritin of 1259 ng/ml (standard value 24-310 ng/ml).

On 30th of August the patient showed increased weight of 6-7 kg, hyponatremia and severe kidney failure, thus hemodialysis was necessary. He received a hemodialysis central venous catheter (CVC) by using the C-arm x ray unit. In the confirmatory xX-ray the CVC was in place, additionally pleural effusion and raised level of diaphragm on the right as well as an enlarged heart and signs of central and pulmonary venous congestion were detected. Hemodialysis was started at the same day without any complications. The following day, the body weight decreased and patient improved. Within the next days the patient as well as laboratory parameters improved and the patient was discharged on 5th of September 2011. He was advised to take Ca-Acetat tid, to follow a kidney-protective alimentation, to take liquid 3-4l/d, to perform a daily weight control, and to stick to an appointment scheduled 3 days later.

Serological examination in the Viennese laboratory showed a positive result for HTNV, which prompted the presence of DOBV infection, given the fact that HTNV is not spread in Europe. This suspect was confirmed by additional serological analysis and PCR test, followed by sequencing (56).

On follow up the patient showed a continuously improvement of laboratory parameters, and on his last appointment on 14th of September 2011, the creatinine

was only slightly elevated to 1, 48 mg/dl, urea and uric acid levels were normal, GFR was 49, 40 ml/min and the pancreatic parameters lowered to 203 U/l of amylase and 219 U/l of lipase. The course of clinic parameters is illustrated in diagram 1. Further investigations were transferred to the general physician. The patient was reappointed for Infectiouse disease consultation in March 2012. Tables 5-9 show laboratory parameters on the course of disease, diagrams 1-6 illustrate the important values of HFRS over the period of time.

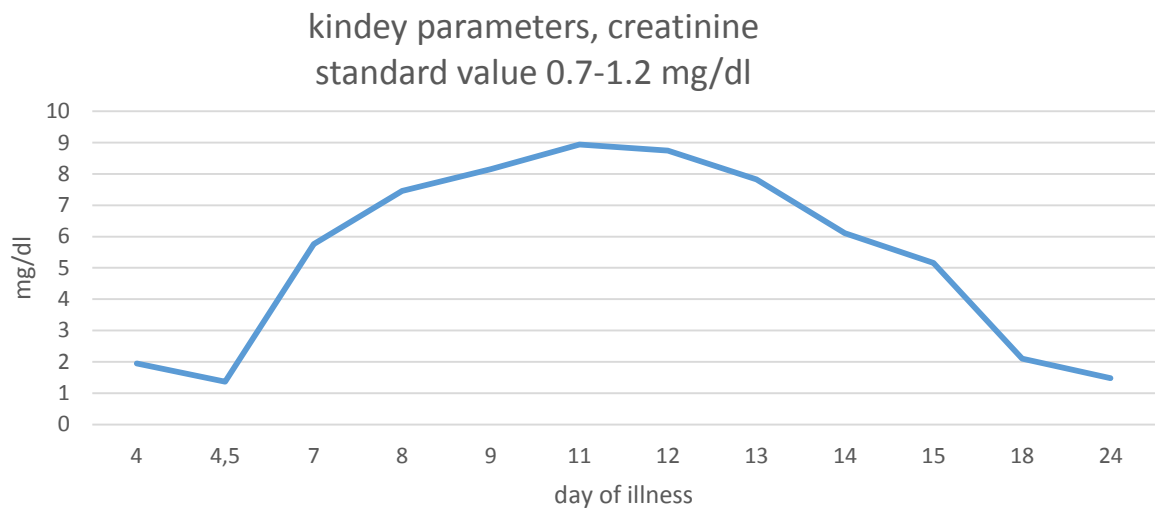

Diagram 1: laboratory parameters: creatinine in the course of disease

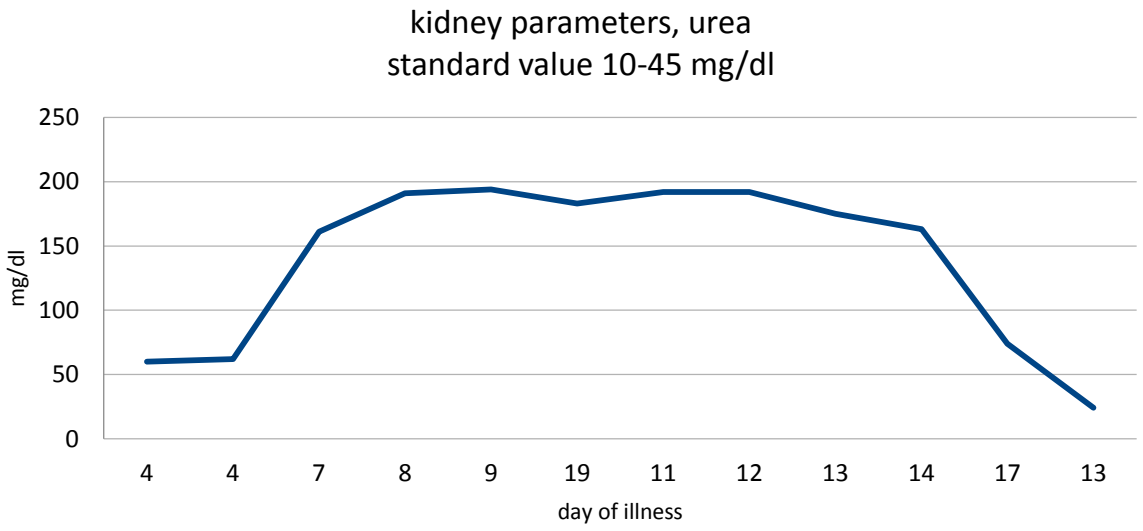

Diagram 2: laboratory parameters, urea in the course of disease

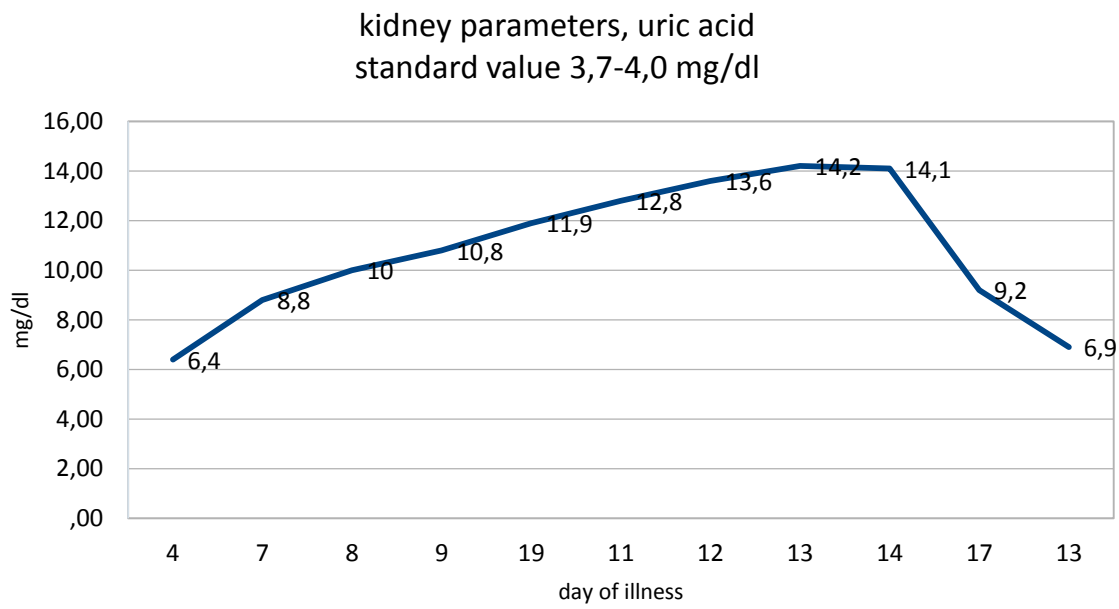

Diagram 3: laboratory parameters, uric acid in the course of disease

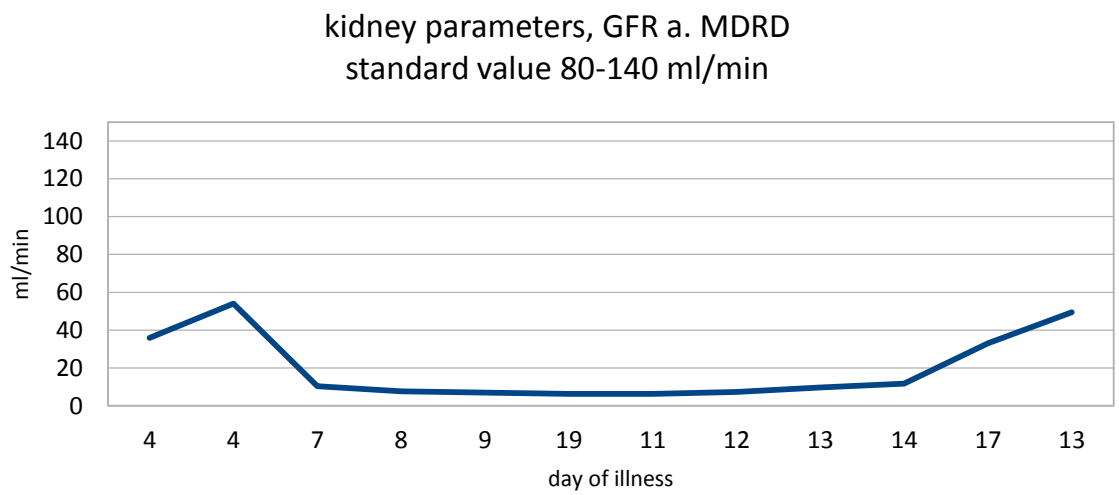

Diagram 4: laboratory parameters, GFR in the course of disease

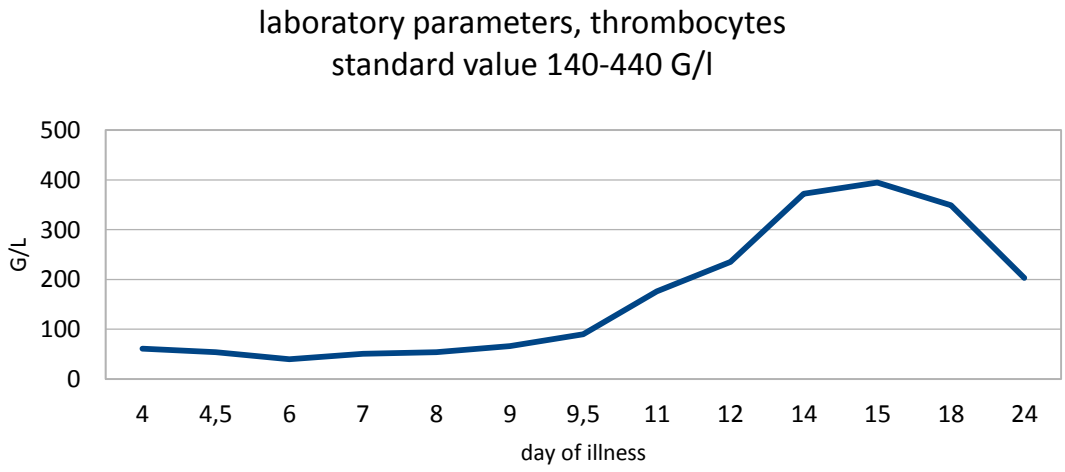

Diagram 5: laboratory parameters, blood count; thrombocytes in the course of disease

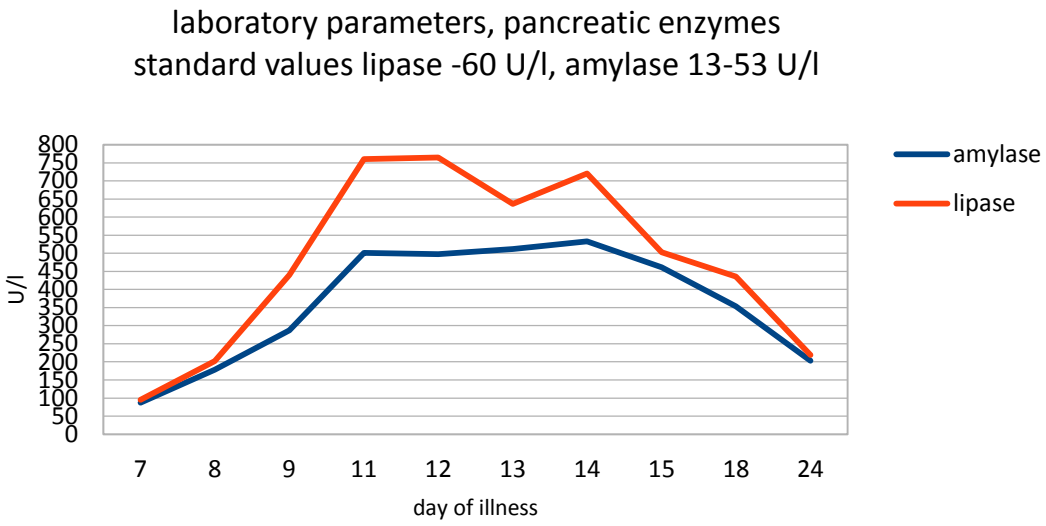

Diagram 6: laboratory parameters, pancreatic enzymes in the course of disease

	day of illness	4	6	8	10	11	13	17
haematology	standard value							
leucocytes	4.4-11-3 G/l	5,72	21,1	10,2	7,76	7,91	6,98	7,03
erythrocytes	4.5-5.9 T/l	6,5	4,9	3,76	3,51	3,87	3,99	4,23
haemoglobin	13-17.5 g/dl	20,5	14,8	11,4	11,9	11,6	11,7	12,6
haematocrit	40-50%	52,8	39,6	30,5	32,2	31,6	33,1	37,3
MCV	80-98 fl	81,2	80,8	81,1	81,3	81,7	83	88,2
MCH	28-33 pg	31,5	30,2	30,3	30,1	30	29,3	29,8
MCHC	33-36 g/dl	38,8	37,4	37,4	37	36,7	35,3	33,8
thrombocytes	140-440 G/l	61	51	66	176	235	372	349
MPV	7.0-13.0 fl			12,4	11,6	10,6	9,7	9,5
differential blood count								
neutrophil granulocytes	50-75 %	76			79	77	78	73
neutrophil gran. abs.	1.8-7.7 G/l	4,4			6,2	6,1	5,4	5,1
eosinophil granulocytes	-5%	0			1	2	2	1
eosinophil gran. abs.	-0.7 G/l	0			0,1	0,1	0,1	0,1
basophilic granulocytes	-1%	1			0	0		
basophilic gran. abs.	-0.2 G/l	0,1			0			
monocytes	2-12 %	7			10	12	8	8
monocytes abs.	0.2-1.0G/l	0,4			0,8	0,9	0,6	0,6
lypmphocytes	20-40%	16	4	10	9	10	12	18
lypmphocytes abs.	1.0-4.8 G/l	0,9			0,7	0,8	0,8	1,3

Table 5: Blood count and differential blood count in the course of disease

electrolytes	Na+	K+	Cl-	Ca 2+ frdP	Ca2+ ges	Mg 2+ ges	phos-phate	phos-phate
standard value	135-145	3.5-5.0	95-110	1.15-1.35	2.2-2.65	0.7-1.1	2.6-4.5	0.84-1.45
unit	mmol/l	mmol/l	mmol/l	mmol/l	mmol/l	mmol/l	mg/dl	mmol/l
day of illness								
4	130	4,5	90		2,33			
4,5	130	4	97		2,18			
6	127	4,7	91		1,91	1,22	6,17	1,99
7	118	4,1	85	0,95	1,78		5,11	1,65
8,3	117	3,5	85	0,88				
8,6	120	3,7	86	0,95				
8,7	123	3,6	87	0,97				
9	123	3,2	89	0,92				
9,5	121	3,4	88	0,91				
10	126	3,9	87		1,84		5,65	1,82
11	130	4	93		1,99	1,23	6,15	1,99
12	130	3,8	95		2,02			
13	137	4,1	100		2,13	1,15	6,32	2,04
14	140	4,4	100		2,26		5,67	1,83
17	144	4,7	107	1,19	2,41	0,68	3,6	1,16
23	141	4,4	102	1,19	2,3	0,7	2,69	0,87

Table 6: electrolytes in the course of disease

	day of illness	4	6	7	8	10	11	13	14	17	23
liver	standard value										
total bilirubin	0.1-1.2 mg/dl			0,51		0,69	0,52		0,6	0,49	0,44
AP 37°C	40-130 U/l		46	52		106	99		90	86	82
GGT 37°C	- 55 U/l	42	17	26		98	80		67	55	44
CHE 37°C	4600-13000 U/l									7757	8815
AST 37°C	-35 U/l	104	75	58		57	35		20	28	36
ALT 37°C	-45 U/l	71	48	44		88	79		52	49	47
heart											
CK 37°C	-170 U/l	137	139	130		78	53	33	26	36	45
CK-MB 37°C	-24 U/l		44	30		15	12	14	11		
LDH 37°C	120-240 U/l	496	525	463		401	373	367	351	306	238
Trop T			26					9			
Myoglobin			269								
coagulation											
PT	70-120%	86	107	113	106	>120	>120	105	103	102	110
PT-INR	INR	1,1	0,98	0,95	0,98	0,88	0,9	0,99	1	1,01	0,97
APTT	26-36 sec	42,1	40,4	31,7	32,9	28,8	29,9	28,5	28	26,9	26,4
fibrinogen	210-400 mg/dl	327	294	311	348	429	439	474	481	357	254
inflammation											
CRP	-5 mg/l	13,7	27,1	21,2	12	13,8	13,3	10	9,2	3,5	1,2

Table 7: laboratory parameters: liver, heart, coagulation and inflammation in the course of disease

blood gas analysis	day of illness	4	8	8,5	8,6	8,7	9	9,3	9,6	17	23
	standard value										
Temp a	°C	37								37	37
Baro a	mmHg	730								727	729
pO2	71-104 mmHg	99,7	34	35	39	39	41	37	46	35,4	41,5
phosphate	7,370-7,450	7,51									
pCO2	35-46	15,7	32	33	33	34	34	37	33	42,2	39,4
akt HCO3	21-26	12,5	17,9	17,8	18,5	19,2	18,2	20,1	18,4	25,9	23
BE	-2 to 3mmol/l	-7	-6,5	-7	-6	-5,3	-6,6	-5	-6,2	1,4	-1,3
BEecf a	-2 to 3mmol/l	-10								1,6	-1,3
sO2 a	95-98,5 %	98								65,2	76,9
pH	7,370-7,450		7,37	7,35	7,36	7,37	7,35	7,35	7,36	7,4	7,39

Table 8: blood gas analysis in the course of disease

urine test	day of illness	6	8	10	10,5	12
strip	standard value					
specific gravity	1,020-1,040	1,013		1,01	1,004	
pH	4,5 – 8,0	7		6	5,5	
leucocytes	/mikrol	0		neg	75	
nitrite		neg		neg	neg	
protein	- 10 mg/dl	400		25	30	
glucose	mg/dl	30		norm		
ketone	mg/dl	neg		neg		
urobilinogen	mg/dl	0,2		norm	0,2	
bilirubin	mg/dl	neg		neg		
Hb/ery	/mikrol	330		50	20	
clarity		clear			cloudy	
colour		yellow			light yellow	
Na+	mmol/l		35		36	47
K+	mmol/l		22		15	7
Cl-	mmol/l		31		33	35
Ca2+ges	mmol/l		0,26		0,24	0,25
Ca2+/cr	-0,60 mmol/mmo		0,05		0,04	0,05
Mg2+	mmol/l		0,92		1,19	0,98
Mg2+/cr	-0,9 mmol/mmo		0,2		0,2	0,2
phosphate	mmol/l		0,7		3,8	5,3
creatinine	mg/dl		64		63	52
urine protein						
protein	-130 mg/l		1580		345	
protein/gcre	-110 mg/gcre		2469		548	
albumin	0-20 mg/l		972		178	
albumin/gcre	0-20 mg/gcre		1519		283	
IgG	-10 mg/l		165		32	
a1MiG	-12 mg/l		29,7		39,3	
a1MiG/cr	-16 mg/gcre		46		62	

Table 9: urine test in the course of disease

4. Results of investigation of captured mice

Between May and June 2012 24 mice were captured. All of the twenty four mice were identified as representatives of either *Apodemus flavicollis* or *Myodes glareolus* species. None was found to inherit any Hantavirus RNA. Neither PUUV nor DOBV RNA was detected by PCR in investigated tissues of captured mice.

5. Discussion

While the study could not verify the presence of DOBV in Styrian yellow necked mice, it is neither approved that the virus doesn't exist within the suspected area. The validity of the field study is very limited due to the small number of caught mice within a relatively narrow time frame and confined area. However, due to limited time and personnel resources it was not possible to extent the time frame and increase the number of captured mice.

In order to assess the question ultimately, a much larger investigation program would be needed. Comparable inquests in other countries as for example Germany, showed DOBV is not widely spread in the primarily host, *Apodemus flavicollis*, and large numbers of animals are needed to be examined so that the virus could be detected. DOBV has been demonstrated to circulate in bordering countries as Czech Republic, Slovakia, Hungary and Slovenia, where cases were sporadically reported and the latter actually being a high endemic area for DOBV infections is neighboring the suspected area of infection. In connection to this investigation another researcher captured mice in other locations of the area suspected to be inhabited with Dobrava-virus infested mice. In the meantime other Dobrava virus infections have been reported from Carinthia and near to Vienna. Investigation of mice from Carinthia were also performed in the Viennese laboratory and confirmed the presence of Dobrava virus in Carinthian *Apodemus flavicollis* (147). Although the presence of Dobrava virus in Carinthia and near to Vienna does not confirm the presence of Dobrava virus in the area near to Graz, it supports the phenomenon that new Hantavirus strains emerge in Austria.

PUUV has been proven to be highly endemic in Styria and every year dozens of confirmed cases are admitted to the University Hospital of Graz. However, no PUUV strain was found in any of the caught mice during the presented study. Not only is

the Hantavirus one representative of emerging zoonoses, but case load and territorial spreading seems to have literally exploded over the past years and HCPS and HFRS will presumably become an important topic that all health care professionals should be aware of.

Even in case the virus has not widely spread in Austrian *Apodemus flavicollis* or other potential carriers within the country to date, the chance for this event to happen eventually will remain valid. There is also the chance of dissemination of other distinct Hantavirus strains of which the primarily host is spread in Austria. Saarema virus has already been found in *Apodemus agrarius* after it was approved in one patient with autochthonous acquired SAAV infection. In addition, with a long incubation period, the import of Hantaviruses from other destinations is always contingent.

DOBV is very rare in Austria and all so far confirmed Austrian Dobrava-virus infections, verified by serological and direct virus detection, manifested mildly with an uncomplicated course and no sequelae (147). In the presented case, the patient also showed mild course and was discharged in good general condition after two weeks of hospitalization. However it is crucial to take heed in this infection and be prepared for a potentially severe course.

In summary, this diploma thesis presents the first case of obviously autochthonous Dobrava virus infection in Styria requiring renal replacement therapy as well as investigation of mice captured in the area of interest and investigated by molecular methods for the presence of Hantavirus. Although our investigation did not show the presence of Dobrava virus in a mice from a certain area near to Graz, the Puumala virus is apparently enzoonotic in Styria as shown by previous investigations. Other Hantavirus strains have recently emerged in Austria (147) and physicians should also be aware of the possibility to be confronted with severe Hantavirus infection to an increasing extent. It cannot be excluded that other not yet endemic and enzootic strains will enter Austria in the near future.

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