UNIVERSITY OF PÉCS

Biological and Sportbiological Doctoral School

Human hantavirus infections in Hungary: occupational safety in risk group and clinical cases in the South-Transdanubian region between 2011-2015

PhD thesis

Miklós Oldal

PÉCS, 2016

UNIVERSITY OF PÉCS

Biological and Sportbiological Doctoral School

Human hantavirus infections in Hungary: occupational safety in risk group and clinical cases in the South-Transdanubian region between 2011-2015

PhD thesis

Miklós Oldal

Supervisor:

Ferenc Jakab

PhD, associate professor

_____ _____

Supervisor Leader of PhD School

PÉCS, 2016

Table of Contents

Abbreviations

AKI – acute kidney injury

ALT – alanine aminotransferase

AST – aspartate transaminase

B-PER – Bacterial Protein Extraction reagent

BSA – Bovine serum albumin

CF - centrifugation

CFR – case fatality rate

CO – cutoff value

DAF – decay-accelerating factor (also known as CD55)

DAB – diamino-benzidine

DOBV – Dobrava-Belgrade virus

EDTA – Ethylenediaminetetraacetic acid

ELISA – enzyme-linked immunosorbent assay

HDPA – High-density particle agglutiantion test

GGT – Gamma glutamyl transferase

HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HFRS – haemorrhagic fever with renal syndrome

HPS – hantavirus pulmonary syndrome

HTNV – Hantaan virus

ICTV – International Committee on Taxonomy of Viruses

IFA – immunofluorescence assay

IFAT – immunofluorescence antibody test

IL – interleukin

INF – interferon

IPTG – Isopropyl β-D-1-thiogalactopyranoside

NE – nephropathia epidemica

NP – nucleocapsid protein

NSP – non-structural protein

NVAV – Nova virus

PBS – phosphate-buffered saline

PBS-T – phosphate-buffered saline plus Tween-20 additive

PCR – polymerase chain reaction

PMSF – phenylmethylsulfonyl fluoride

POS – post onset of symptoms

PUUV – Puumala virus

rRT-PCR – Real time RT-PCR

RT – reverse transcription

SAAV – Saaremaa virus

SDS – sodium- dodecyl sulfate

SEOV – Seoul virus

SWSV – Seewis virus

TBS – TRIS-buffered saline

TBS-T – TRIS-buffered saline plus Tween-20 additive

TRIS – tris-(hydroxi-methil)-amino methane

TULV – Tula virus

WB – Western blot

1. Introduction

Among human pathogenic infections, zoonoses can be held responsible for an enormous part of disease cases. Zoonoses are infectious animal diseases (usually of vertebrates) that can be naturally transmitted to humans. Zoonotic agents represent a range of disease pathogens such as viruses, bacteria, fungi and parasites. According to a 2001 study, out of the roughly 1.400 pathogens known to infect humans, around 60% are zoonotic (Taylor et al., 2001). Hence, some examples of the world's best-known diseases are also caused by zoonotic agents, such as: malaria, influenza, yellow fever, rabies, or the infamous modern-world Ebola virus, which just got its largest ever outbreak in West-Africa in 2015.

There are several factors nowadays that drive the emergence and/or re-emergence of zoonotic agents. The most important factor is the growing human population, which results in further issues i.e. increased urbanization, land use and consequential deforestation. Growing human population also induces changes in both food processing as well as in the distribution of animal/vector populations. Modern technology enables us to travel long distances in short periods, often to "exotic" areas, where local infectious agents have a relatively small endemic area but are highly pathogenic. Finally, there are factors like environmental and climate changes, which are at least partly independent from human activities. All the above mentioned added up and result in the generally increasing overlap of animal and human habitats, raising a high chance of disease transmission.

Hantaviruses are the focus of the current work due to them being one of the significant zoonotic agents, not only on a worldwide scale but also in Hungary. These pathogens can infect anyone without gender or age restriction, and while human cases are not the most prominent on an annual scale, recognized infections often have a severe course. Hantavirus research is done by very few in Hungary, with little data regarding the country-wide distribution of the causative agent as well as the characteristics and prevalence of clinical cases. The present work tries to provide an insight into the current status of hantavirus infections in Hungary.

2. Literature review

2.1 History of hantaviruses

A disease highly similar to Haemorrhagic fever with renal syndrome (HFRS) – a type syndrome caused by hantaviruses was first described by a Chinese medical guide called Whang-Jae-Nae-Kyung in 960 AD (Lee, 1982a). However, only about 1000 years later, hospital records from Far-East Russia mentioned the next written descriptions of the disease in 1913. Since 1932, HFRS became generally known as nephroso-nephritis by Russians and as Songo fever by Japanese military physicians. In Europe, two Swedish physicians Myhrman and Zetterholm simultaneously reported a mild HFRS-like disease known as nephropathia epidemica (NE) in 1934 (Myhrman, 1951).

Throughout all its history, HFRS has often been associated with military operations. This is not surprising, given that during wartime conditions, military personnel's movements greatly overlap with different rodent habitats. Cases of "trench nephritis" during the First World War have been likely caused by hantavirus infections.

Amur River forms the border between the Russian Far East and North-eastern China. In the early 1940s, it was this place where the Japanese Army suffered approximately 12,000 cases of what was undoubtedly HFRS. In the Second World War, HFRS reached such a high prevalence that intense efforts were made to identify the causative agent. Sadly, these efforts sometimes included wicked human experimentation in Russia, Japan and China where volunteer prisoners of war were injected with filtered blood and urine from HFRS patients. It was demonstrated that the disease was indeed transmissible by a micro-organism and that it could be propagated in humans (Gajdusek, 1982; Lee, 1982a, b, Vaheri, 2008). Despite the numerous military-related cases, it was during the Korean War between 1951 and 1954, when HFRS received much more attention: 3200 UN soldiers contracted HFRS, then-known as Korean haemorrhagic fever. Western physicians became familiar with HFRS, and this started a new wave of efforts in identifying the etiologic agent of the disease. Professor Ho Wang Lee was funded a research grant to perform studies that eventually lead to the discovery of Hantaan virus (Lee et al., 1978). As identification of the causative agent from acutely ill patients proved unsuccessful, Professor Lee

joined forces with Karl M. Johnson and focused on rodents in the rural zones where human disease occurred with regularity (Johnson, 2004). Finally, the viral antigen was detected by indirect immunofluorescence when convalescent patient sera were used on acetone-fixed lung sections of *Apodemus agrarius* mice (Lee et al., 1978). Infected rodent lung samples were sent to the US Army Laboratory at Fort Detrick (USAMRIID) where a virus was isolated in cell cultures (French et al., 1981). The virus was named Hantaan virus after the Hantaan River that runs between South and North Korea near Songnaeri where Ho Wang Lee had a field station. US investigators were able to propagate the virus in cultured cells, revealing that Hantaan virus had a morphology and genetic content similar to viruses in the family Bunyaviridae (French et al., 1981; McCormick et al., 1982; Schmaljohn and Dalrymple, 1983; Schmaljohn et al., 1983; White et al., 1982).

Meanwhile in Finland, Puumala virus (PUUV), the causative agent of nephropathia epidemica was discovered in bank voles (*Myodes glareolus*), applying the same methodology (Brummer-Korvenkontio et al., 1980). Other HFRS-causing pathogens were soon also discovered: Seoul virus in 1982 from brown rats (*Rattus norvegicus)* in Korea (Lee et al., 1982), Dobrava-Belgrade virus (DOVB) from yellow-necked mice (*Apodemus flavicollis*) in Slovenia and Serbia in 1992 (Avsic-Zupanc et al., 1992; Gligic et al., 1992) and Saaremaa virus (SAAV) from Estonian field mice (*A. agrarius*) in 1997 (Plyusnin et al., 1997). Saaremaa virus is antigenically closely related to Dobrava-Belgrade virus but differs from it in other respects, including its rodent host and its reduced virulence for humans (Klempa et al., 2013; Plyusnin et al., 2006). Recently, a scientific debate arose, some researchers claiming that it SAAV is in fact a subspecies of DOBV. It was proposed that DOBV should be subdivided into four related genotypes – Dobrava, Sochi, Kurkino and Saaremaa – that have characteristic differences in their phylogeny, specific host reservoirs, geographical distribution, and pathogenicity for suck-ling mice and humans (Klempa et al., 2013). These closely related hantavirus genotypes/species cause either life-threatening (the Dobrava and Sochi genotypes), relatively mild (the Kurkino genotype) or possibly unapparent (the Saaremaa genotype) human infections.

Detailed antigenic and molecular studies of HFRS-associated and related hantaviruses led to the conclusion that hantaviruses could not be classified with any other known group of viruses in the family Bunyaviridae (Avsic-Zupanc et al., 1992;

Schmaljohn and Dalrymple, 1983; Schmaljohn et al., 1983, 1985). Consequently, a proposal was made to the International Committee on the Taxonomy of Viruses (ICTV) to establish the Hantavirus genus, and this proposal was approved in 1994.

Since then, the number of newly identified hantavirus species is rapidly growing. Holmes and Zhang in a most recent review hypothesize that our current picture on hantavirus biodiversity is just the tip of ice berg. As of January, 2015, there are 61 species enrolled into the Hantavirus genus, and numbers are expected to be growing further (Figure 1, Holmes and Zhang, 2015).

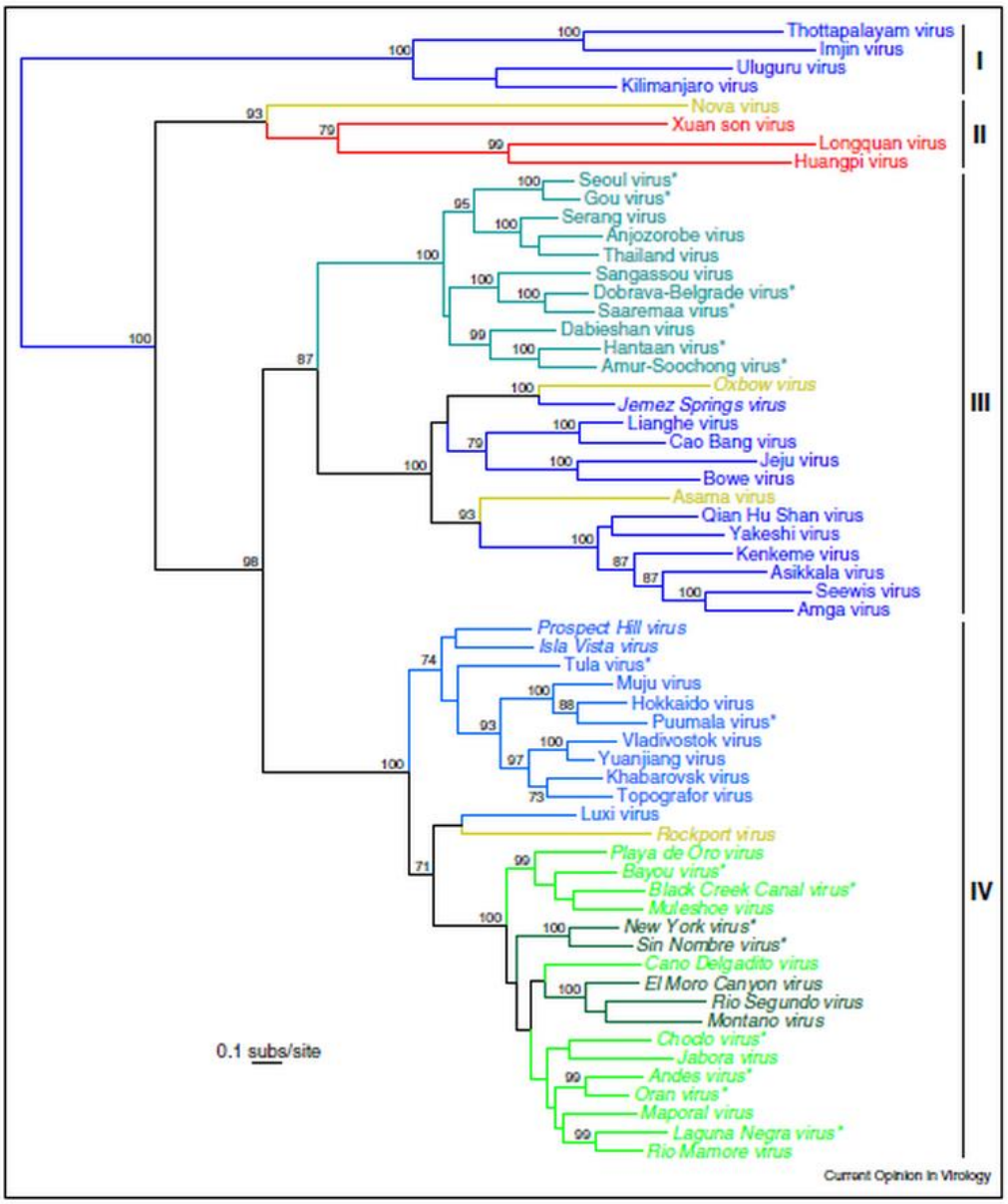

Figure 1. *Phylogeny of hantavirus based on available coding regions of the S segment (taken from GenBank) and inferred using the maximum likelihood method available in PhyML (version 3; Ref. 66), excluding poorly aligned regions. Colors represent the primary host species groups as follows: Chiroptera (bats) = red; Soricidae (shrews) = dark blue; Talpidae (mole) = yellow; Murinae (Old World mice and rats) = turquoise; Arvicolinae (voles and their relatives) = cobalt; Sigmodontinae (New World mice and their relatives) = lime; Neotominae (New World mice and rats) = dark green. Viruses from the New World are shown in italics, and those with evidence of human infection are marked with a * symbol. The numbers above or below branches indicate bootstrap values >70%. The four phylogroups (I-IV) are marked. The scale bar represents the number of nucleotide substitutions per site.*

2.1.1 Hantaviruses in Europe

In Europe (excluding Hungary), the following hantavirus species have confirmed presence (in the order of importance regarding the number of human cases): PUUV, DOBV, Tula virus (TULV), Seewis virus (SWSV), and Asikkala virus (ASIV). TULV was first detected in common vole (*Microtus arvalis)* and from East European vole (*M. levis)* in Russia (Plyusnin et al. 1994). Later, TULV had been identified several other countries, i.e. Austria, Belgium, Germany, Netherlands, Poland, Slovakia, as well as in the former Yugoslavia. (Bowen et al., 1997, Heyman et al., 2002, Reusken et al., 2008, Sibold et al., 1999, Song et al., 2004). Despite its fairly widespread distribution and a high prevalence of anti-TULV antibodies in humans in Germany (Mertens et al, 2011), TULV has only been associated with human disease in some specific cases (Klempa et al., 2003, Zelená et al., 2013). In the mid-2000s, hantavirus research turned towards other host species besides rodents, shedding light to fact that members of the order Soricomorpha (shrews and moles) may also serve as reservoir hosts for newly discovered hantavirus species. Seewis virus (SWSV) was detected in lung tissue samples of Eurasian common shrew (*Sorex araneus*) in Switzerland (Song et al., 2007). Asikkala virus (ASIV) is one of the latest discovered hantaviruses, it was identified first in Finland (Sironen et al., 2010) and lately in the Czech Republic from pygmy shrews (*Sorex minutus*) (Radosa et al., 2013). The pathogenic relevance for SWSV, NVAV and ASIV has yet to be determined, as no human diseases associated with the latter viruses are reported thus far.

2.1.2 Hantaviruses in Hungary

It was Scharninghausen et al. who first described hantaviruses in the country in 1999. Researchers trapped animals near the village of Taszár (a local airbase also used by NATO forces); RNA of a virus that was phylogenetically most closely related to DOBV had been detected from field mice (Scharninghausen et al., 1999). The first larger-scale study processed data collected between 1992 and 2000. Nearly 20 different mammalian species were tested by serological methods. Results revealed the prevalence of antibodies to human pathogen hantaviruses among rodents of about 7.25 %. Direct nucleic acid detection became part of the research toolset and showed that PUUV and DOBV species exist in the country (Ferenczi et al, 2003, Ferenczi et

al., 2005). DOBV was identified as predominant hantavirus species in the Transdanubian region (Jakab et al., 2007/a). Additionally, it was suggested that the actual prevalence of DOBV is underestimated, due to the fact that simultaneous use of molecular and immunological detection is rare, although the two methods provide significantly more accurate results (Németh et al., 2011). TULV was first identified in 2008, out of 46 common voles captured near the south-western border, 17 tested positive for TULV RNA by PCR (Jakab et al., 2008). A year later, the parallel circulation of PUUV, DOBV and SAAV was also published (Plyusnina et al, 2009). That time, SAAV was considered a separate species, however, later this proved to be the basis of fierce taxonomical debates mentioned earlier, leading to establishing SAAV as a DOBV subspecies, with local supporting contributions (Németh et al., 2013). As an example of extended hantavirus research towards non-rodent host species, Nova virus (NVAV) was discovered near a Hungarian village bearing the same name. The virus was detected from liver tissues of the common mole (*Talpa europea*) (Khang et al., 2009). In a more recent study from Serbia, DOBV hantavirus was genetically detected in the edible dormouse (*Glis glis*). Phylogenetic analysis of the obtained sequences implies putative DOBV spill-over infection of the single specimen of *G. glis* from *A. flavicollis*. (Stanojevic et al., 2015).

Next to investigations into hantavirus host species in the Hungary, clinical studies also existed. The first official documentation of HFRS cases in Hungary originate from a 1971 publication (Trencséni and Keleti, 1971). It was still seven years before the identification and classification hantaviruses (Lee at al. 1978), hence apart from the occurrence of the disease, no further details were mentioned. Between 1987 and 1993 the Hungarian Army Medical Corps confirmed HFRS in 55 patients using IFAT, high density particle agglutination and ELISA techniques (Faludi and Ferenczi, 1995). A couple of years later, in a national serosurvey conducted in 2000, more than two thousand citizens above 20 years of age were tested for antibodies against hantavirus. Results indicated that about 10% of healthy people can be considered seropositive. Affected persons lived in all areas of the country (Ferenczi et al., 2003, Ferenczi et al., 2005).

Parallel immunological and molecular findings confirmed a DOBV infection for the first time in a 46 year-old hunter, from the Southwestern region of the country (Jakab et al, 2007/b).

The fact that hantavirus infection may be hard to diagnose was also supported by a Hungarian study. In one reported case, a 27 year-old man with fever, nausea, vomiting, bloody diarrhoea and severe abdominal pain localized mainly at the right lower quadrant of the abdomen was admitted to hospital. Based on the latter findings supported by computerized tomography, acute perforated appendicitis was suspected and an explorative laparatomy was performed. The operation, however, did not confirm the diagnosis (Jakab et al., 2011).

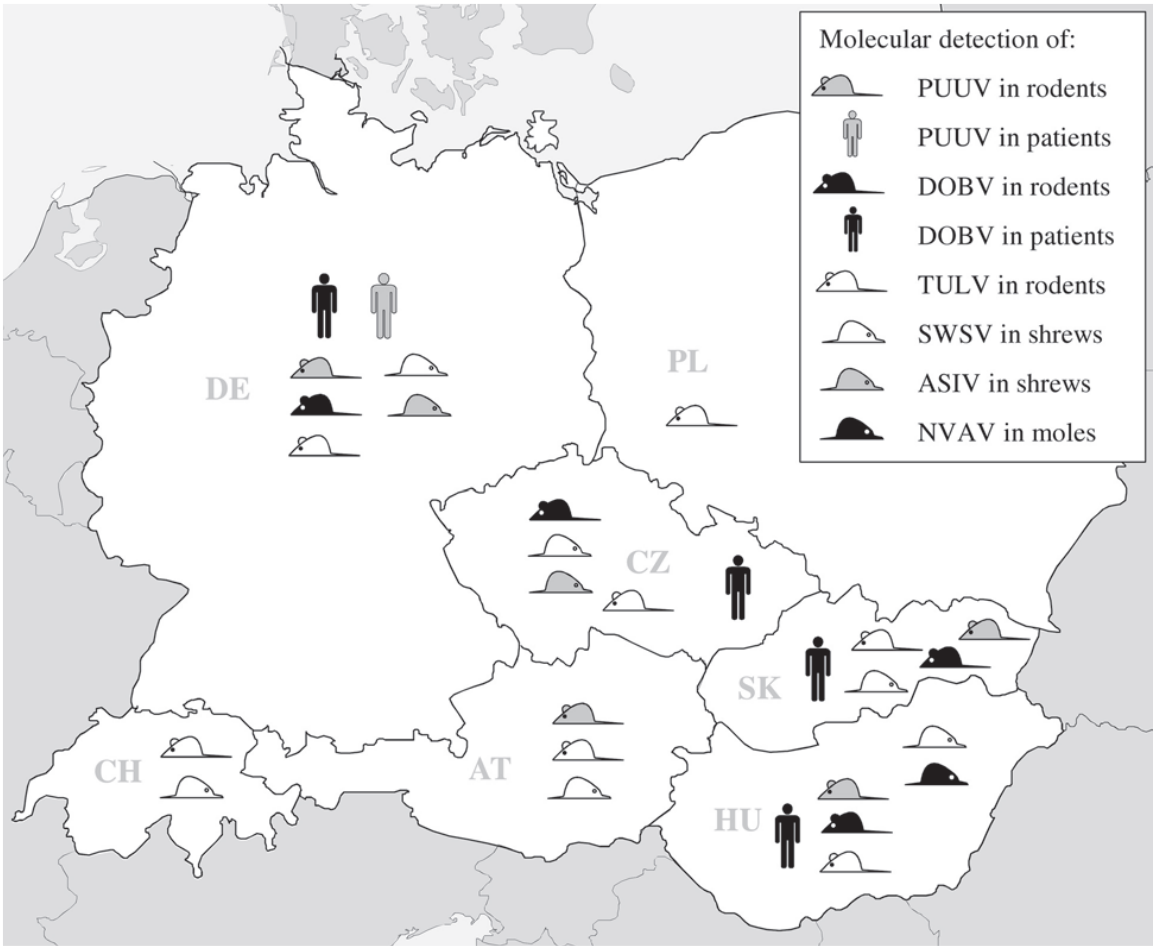

Figure 2. *Summarizing map of Central Europe indicating countries with molecularly documented presence of hantaviruses (Klempa et al., 2013). AT, Austria; CH, Switzerland; CZ, Czech Republic; DE, Germany; HU, Hungary; PL, Poland; SK, Slovakia; ASIV, Asikkala virus; DOBV, Dobrava- Belgrade virus; NVAV, Nova virus; PUUV, Puumala virus; SWSV, Seewis virus; TULV, Tula virus.*

2.2 The general characteristics of Hantaviruses

Hantaviruses are members of the *Bunyaviridae* family, which is the largest RNA virus family. *Bunyaviridae* is comprised of more than 350 viruses divided into five genera

based on serological, morphological, and biochemical characteristics: *Orthobunyavirus*, *Nairovirus*, *Phlebovirus*, *Hantavirus*, and *Tospovirus* with additional unclassified viruses (McElroy Horne, and Vanlandingham, 2014). Viruses within the Bunyaviridae family, (often simply referred to as Bunyaviruses) all have single-stranded, negative sense or ambisense, tripartite RNA genomes. Viral genes are placed on one of three segments: the negative sense large (L) segment, coding for the RNA-dependent RNA polymerase for transcription and replication; the negative sense or ambisense (*Tospovirus*) medium (M) segment, which encodes the Gn and Gc viral glycoproteins and a non-structural protein (NSp) of unknown function (possibly virulence), and the small (S) segment, coding for the N nucleocapsid protein and in some genera a non-structural protein (NSs) that may function in the innate immune response, in overlapping or non-overlapping reading frames (Bishop and Beaty, 1988, Flick and Bouloy, 2005, Walter and Barr, 2011).

Hantaviruses have a virion of 80-120 nm in diameter and the genome itself consists of approximately 12.000 nucleotides. Nucleocapsid proteins (NPs) interact with the S, M and L segments of the viral genome to form helical structures. Virus-encoded RNA polymerase is placed in the interior. Genome segments are encapsulated by a 5 nm-thick lipid bilayer (viral envelope). The viral surface proteins Gn and Gc are embedded within the lipid bilayer.

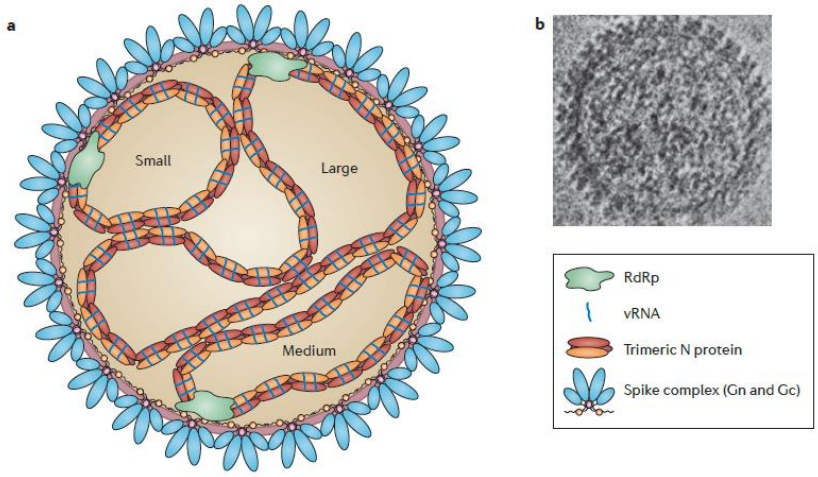

Figure 3. *Hantavirus particles, genes and proteins (Jonsson et al., 2010). a | Schematic representation of the hantavirus virion. The hantavirus particle contains the trisegmented viral RNA (vRNA) genome, comprising the small, medium and large ORFs. These are encapsidated by nucleocapsid (N) protein. The outer part of the virion consists of spikes*

comprising four units of each glycoprotein, Gn and Gc. The viral genome is replicated and transcribed by RNA-dependent RNA polymerase (RdRp). ***b*** *| A hantavirus particle viewed by cryoelectron microscopy. The spike height is invariably 12 nm, and the median diameter of the virion is 135 nm. Part* ***b*** *image is courtesy of P. Laurinmäki and S. Butcher, University of Helsinki, Finland.*

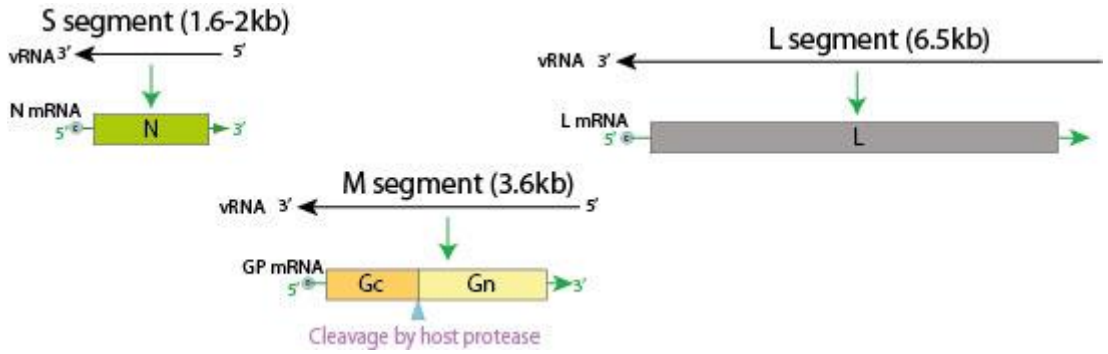

Figure 4. *Genome structure of hantaviruses. Segmented Negative-stranded RNA linear genome, L segment is between 6.8 and 12 kb, M segment between 3.2 and 4.9 kb and S segment between 1 and 3 kb. Encodes for four to six proteins, Some hantaviruses also encode a non-structural protein on their S segment (ViralZone - http://viralzone.expasy.org/).*

2.3 The pathology of hantavirus infection

Hantavirus infections have a complex epidemiology and clinical course. Pathogenicity largely varies for the different hantavirus species. Men are more affected than women, which fact can be probably linked to behavioral factors. (Krautkrämer et al., 2013). However, case fatality rates (CFRs) are higher for women, based on reports from different countries (Hjertqvist et al., 2010; Klein et al., 2011; Martinez et al., 2010). Thus far, there is no information why CFR is higher for women. Disease outcome has a broad spectrum ranging from asymptomatic infection to severe cases and courses does not seem to depend on gender-specific differences since laboratory parameters, renal, cardiac or pulmonary findings are similar in both sexes. In contrast, Puumala virus (PUUV) infection causing severe central nervous system complications is more prevalent in young male patients. (Hautala et al., 2011).

Hantavirus disease demonstrates various symptoms and may affect different organs. Endothelial and epithelial cells of several organs as well as immune cells represent targets of hantavirus infection. Identification of target cells and the

characterization of replication steps and its effects on cellular function play an important role in the understanding of pathogenesis of hantavirus infection. Entry and spread of pathogens are major determinants in pathogenicity. The abundance and use of receptors determine the susceptibility of target cells. The use of certain receptors may also activate signaling pathways and thereby contributing to cellular dysfunction. The localization of receptors at the basolateral face of polarized cells may require the crossing of the monolayer and is often associated with the disruption of epithelial and endothelial barriers. In vitro analysis of susceptibility and permissiveness identified different cell types as target cells for hantaviruses. Human cell lines of kidney, lung, liver, primary renal cells and peripheral blood monocytes/macrophages were permissive for hantavirus infection (Guhl et al., 2010; Krautkrämer et al., 2011; Raftery et al., 2002; Temonen et al., 1993). Viral antigen is detectable in epithelial cells of different organs of patients with hantavirus infection (Groen et al., 1996; Hautala et al., 2002; Hung et al., 1992; Kim et al., 1993). Endothelial and epithelial cells represent highly specialized cells and differ enormously between different organs. The determinant of organ manifestation of hantavirus disease are not completely understood. The organ manifestation of different pathogenic hantaviruses is not restricted to a certain organ as demonstrated by cases of PUUV infection with cardiopulmonary involvement or central nervous system complications (Gizzi et al., 2013; Hautala et al., 2002, 2010; Kanerva et al., 1996; Rasmuson et al., 2011, 2013).

Differences in the receptor usage do not seem to be responsible for the variation in organ tropism. Pathogenic hantaviruses causing HFRS or HPS enter cells via integrin αvβ3 and non-pathogenic viruses use integrin β1 (Buranda et al., 2010; Gavrilovskaya et al., 1998, 1999; Klempa et al., 2012; Krautkrämer and Zeier, 2008). In addition, the entry of hantaviruses is mediated by CD55 (Krautkrämer and Zeier, 2008). Protein gC1qR/p32 was also described to mediate hantavirus entry (Choi et al., 2008). CD55 and gC1qR/p32 are components of the complement system. Whether the entry via these receptors interferes with their function within the complement cascade and contributes to hantaviral pathogenesis is so far not elucidated.

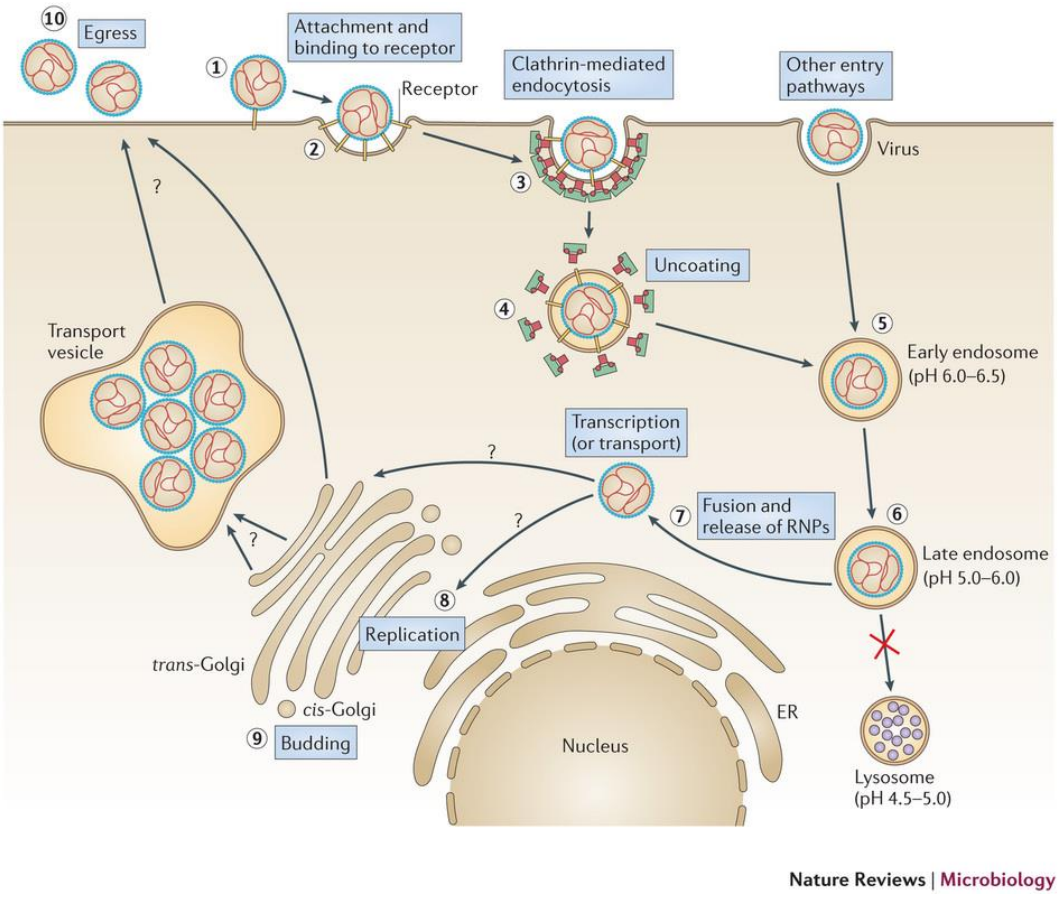

Figure 5. *The life cycle and replication of hantaviruses. The hantavirus virion attaches to a receptor on the cell surface (step 1). This binding event induces endocytosis signaling (step 2), after which the virion enters the cell in clathrin-coated vesicles (step 3). Other entry pathways have also been observed for some hantaviruses. In the case of clathrin-mediated endocytosis, the clathrin coat of the vesicle is disassembled (step 4), and the virion-harbouring vesicle enters the early endosome (step 5), which matures into a late endosome (step 6). Fusion between the viral and endosomal membranes is driven by acid-induced conformation changes in the viral fusion protein in the late endosome. This results in release of the viral ribonucleoproteins (RNPs) (step7). Initial transcription might take place at the site of release; alternatively, the RNPs might be transported to the ER–Golgi intermediate compartment (ERGIC) for transcription. It is also possible that the virus is directly transported to the Golgi complex from the late endosome, either before or after fusion. Viral replication is thought to occur in viral factories that might be located at the ERGIC or the cis-Golgi (step 8). The nascent viruses are thought to bud into the cis-Golgi (step 9), from where they are transported to the plasma membrane for release, presumably via recycling endosomes. The egress of progeny virions takes place at the plasma membrane (step 10), (Vaheri et al. 2013).*

2.4 The clinical picture of hantavirus infection

When speaking about disease course and clinical signs, we have to make a distinction between two characteristic clinical pictures, namely "Haemorrhagic Fever with Renal Syndrome" (HFRS) and "Hantavirus Pulmonary Syndrome" (HPS). According to the traditional views, HFRS is caused by viruses circulating in the Eurasian continent, i.e. "Old World Hantaviruses", whereas HPS is caused by viruses endemic in both the North-and South American continent, i.e. "New World Hantaviruses." This so-called "transatlantic schism" has however been challenged recently, due to the fact that there is an increasing number of case reports, where the clinical course of HFRS and HPS are mixed. In European HFRS caused by Puumala virus (PUUV), pulmonary symptoms with clinical findings of cough, dyspnoea, interstitial lung infiltrates, pleural effusion, and impaired pulmonary function were recorded (Kanerva et al., 1996; Linderholm et al., 1992; Linderholm et al., 1997). Prominent lung involvement in HFRS has also been reported (Caramello et al., 2002; Clement et al., 1994; Hoier et al., 2006; Seitsonen et al., 2006). These observations drove several scientists and physicians to suggest that HFRS and HPS are actually the different manifestations of the same disease, and instead of the two clinical pictures, only a single term called "hantavirus disease" should be used (Clement et al., 2012; Rasmuson et al., 2011). While these suggestions may be correct in many ways, we stick to the original differentiation of HFRS and HPS, since there is still no official statement on the newly suggested views.

2.4.1 Haemorrhagic fever with renal syndrome (HFRS)

Similarly to the vast majority of viral infections, a HFRS also starts with non-specific (or "flu-like") symptoms, including headache, muscle or joint pain, nausea, fever, sore throat, and general lethargy. In more than 50% of cases, the illness does not progress to the further clinical stages, thus one can fully recover without even noticing that there had actually been a hantavirus infection. In typical cases, however, the disease progresses into further five stages:

1. Febrile phase: next to the introductory non-specific symptoms, abdominal pain, diarrhea, chills, sweaty palms, redness of the cheeks and nose can also occur. Symptoms arise from 2-3 days post exposure and last up to 2-3 weeks.

2. Hypotensive phase: platelet counts start to drop resulting thrombocytopenia, the most typical laboratory finding, together with tachycardia and hypoxemia. Hypotensive phase lasts two days.
3. Oliguric phase: renal failure and proteinuria and/or haematuria manifest for 3-7 days.
4. Diuretic phase: renal failure shifts to diuresis of 3-6 liters per day and lasts from a couple of days up to three weeks.
5. Convalescent phase: progressive improvement of laboratory parameters and patient status, full recovery may last several weeks. In rare extreme cases, permanent kidney damage can be acquired.

HFRS has a case fatality rate of up to 12%, depending on the causal agent (DOBV being responsible for the highest CFR).

2.4.2 Hantavirus pulmonary syndrome (HPS)

As in HFRS, prodromal symptoms include flu-like symptoms such as fever, cough, myalgia, headache, and lethargy. In contrast to HFRS, HPS does not have several characteristic stages, but after the prodromal phase, a sudden onset of dyspnea occurs, which rapidly progresses into pulmonary edema that is often fatal despite mechanical ventilation and intervention with potent diuretics. HPS has a case fatality rate of 36%. Even though the mortality rate is higher for HPS than for HFRS, only a few thousand HPS cases have been detected in the past 20 years, whereas approximately 50,000 HFRS cases are estimated to still occur worldwide annually.

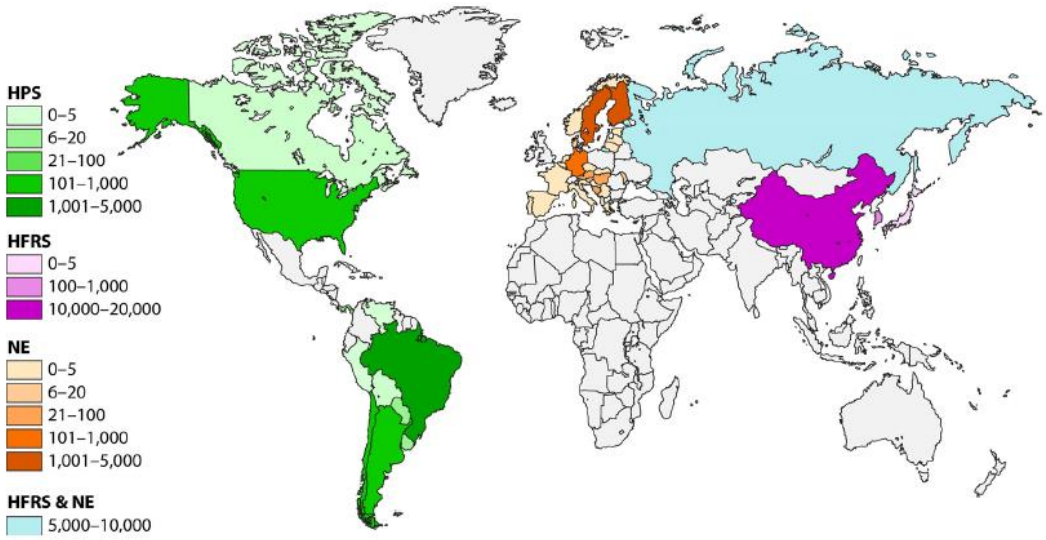

Figure 6. *Geographical representation of approximate hantaviral disease incidence by country per year. (Courtesy of Douglas Goodin, Kansas State University; reproduced with permission.) (Jonsson et al., 2010)*

2.5 Laboratory diagnosis

2.5.1 Serological tests

Upon the onset of symptoms, virtually all acute HFRS and HPS cases have IgM and IgG antibodies to the N protein. Hence, serological tests that detect IgM and/or IgG antibodies

against hantaviral antigens in serum are the most common approaches for the laboratory diagnosis of suspected cases of HPS and HFRS. One of the first serological tests used for

diagnoses of HFRS in Europe and Asia was the indirect immunofluorescence assay (IFA) using hantavirus-infected cells fixed as an antigen on microscope slides. The use of virus-infected cells for serological tests is not widely used because cell culture infections require BSL-3 and BSL-4 laboratories. Thus, most hantavirus antigens currently used in serological tests are those derived by using recombinant DNA methods. These antigens are mostly N proteins, but Gn and Gc proteins have also been produced. The N protein has been expressed and purified from a number of recombinant expression systems, including bacterial (Jonsson et al, 2001, Kallio-

Kokko et al., 2000), baculovirus (Kallio-Kokko et al., 2000, Schmaljohn et al., 1988), insect (Vapalahti et al., 1996), Saccharomyces spp. (Razanskiene et al., 2004, Schmidt et al., 2005), plant (Kehm et al, 2001, Khattak et al., 2002), and mammalian (Billecocq et al., 2003) cells. All three structural proteins (Gn, Gc, and N) can induce a high level of IgM detectable at the onset of symptoms (Brummer-Korvenkontio et al., 1980, Elgh et al., 1997, Figueiredo et al., 2008, Lundkvist et al., 1993 Vapalahti et al., 1995), but the IgG response to the glycoproteins may be delayed, and in the acute phase, the diagnostic IgG IFA pattern is granular (Kallio-Kokko et al., 2001). The N protein, the most abundant viral protein, induces a strong humoral immune response in humans and rodents and shows three major epitopes of cross-reactive antigens for hantaviruses. Numerous studies have demonstrated that these antigenic sites are located in the amino- proximal region of the N protein (Lindkvist et al., 2007, Lundkvist et al, 1996, Prince et al., 2007). The N protein is suitable for use as an antigen in immunoenzymatic assays (EIAs) for the diagnosis of hantavirus infection (Figueiredo et al., 2008, Hjelle et al. 1997) as well as strip immunoblot tests (Hjelle et al., 1997). However, the most common serological tests for hantaviruses are indirect IgG and IgM enzyme-linked immunosorbent assays (ELISAs) as well as IgM capture ELISAs.

2.5.2 Molecular diagnostics

The hantavirus genome can be rapidly detected by reverse transcription-PCR (RT-PCR) with clinical samples, such as blood, serum, or organ fragments, from the first day after the onset of illness. The detection of viral genomes in patients before the first day of symptoms has been reported (Ferres et al., 2007, Padula et al. 2007). In general, primary amplification of hantaviral RNA from cell culture can be performed by using RT-PCR (Giebel et al., 1990, Hjelle, 1998). 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 (Heiske et al. 1999, Dzagurova et al., 2009).

At a comparatively high cost, TaqMan PCR systems are rapid and highly sensitive, coupled with real-time assaying, eliminating the need for agarose gel visualization. Hantavirus-specific TaqMan PCR assays usually target the S segment (Aitichou et al., 2004, Weidmann et al. 2005, Lagerqvuist et al. 2016).

3. Aims of the study

1.) Producing PUUV nucleocapsid protein using *Escherichia coli* expression system.

2.) Optimization of an ELISA test using the newly produced antigen for large-scale sample screening.

3.) Optimization of a Western-blot method using the newly produced antigen for acute-case hantavirus diagnostics and for the confirmation of ELSIA-postives.

4.) Testing of acute-case hantavirus patients by Western blot method and PCR.

5.) Molecular characterization of those viruses detected by RT-PCR from patient samples.

6.) The ELISA screening of hunters' and forestry workers' blood samples collected between 2011 and 2013 and confirming of results by Western blot analysis.

7.) The construction of a "risk group hantavirus seroprevalence map" of Hungary based on the results of ELISA screening and western blot confirmation.

8.) The assessment of hantavirus prevalence among patients hospitalized with AKI in the region, in cooperation with the following clinics of the University of Pécs:

1st Department of Internal Medicine, Department of Infectology, and the 2nd Department of Internal Medicine and Nephrology Centre.

4. Materials and methods

4.1 Human samples and volunteers

Among the topics of the thesis we differentiate two main groups of samples. One group is composed of acute patient samples, these were provided within the frames of cooperation with the University of Pécs, the 1st Department of Internal Medicine, Department of Infectology, and the 2nd Department of Internal Medicine and Nephrology Centre. Sera were collected prospectively from persons hospitalized with acute kidney injury (AKI) between January of 2011 and December of 2015, in the University Hospital of Pécs. Patients were selected based on the following criteria: serum creatinine concentration higher than 125 μmol/l upon hospital admission, which showed a 1.5-fold increase during a seven-day period; moreover, patients had fever, high leukocyte count, thrombocytopenia, elevated transaminase levels, as well as urinary abnormalities as parameters that raised the possibility of hantavirus infection. Sera were obtained from native blood samples immediately after arrival, and stored at -20°C. Epidemiological and clinical data were recorded in the time of hospital admission and throughout the hospital stay.

The other group of samples is composed of blood sera collected from volunteer hunters and forestry workers between June 2011 and February 2013 at several sampling sites across the country, sites are indicated in Figure 9. All volunteers provided a signed consent and were asked to fill out a questionnaire, containing the following aspects: epidemiological data (i.e.: age, place of birth and living), exact work duties, work time in forestry and on-field, previous zoonotic infections, animal contacts, domestic animals kept, medication received as well as drugs taken. Factors in context of possible hantavirus preludes were also recorded, i.e. unclarified severe renal complaints and diseases with high fever. Study was approved by the National Medical Scientific Ethical Board (ETT-TUKEB No#2213-0/2010-1018EKU).

4.2 Viral RNA extraction from animals, tissue culture, and human samples

Viral RNA of DOBV originated from naturally infected rodents, captured in the Haljevo forest, near the municipality of Gola in Croatia. Viral RNA of DOBV and PUUV was extracted using TRIzol® (Invitrogen) reagent, according to the

manufacturer's recommendations. In case of DOBV, lung tissue samples of naturally infected yellow-necked mice were homogenized in 500 ml PBS (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.46 mM KH_2PO_4, pH 7.4), After CF for 10 min, at 4°C, 14.000 g, 100 µl supernatant was added to 500µl of TRIzol® reagent, followed by incubating samples for 10 min at room temperature. After addition of 100 µl of chloroform (Sigma), samples were incubated for another 10 min. After CF for 15 min at 4°C, 16.000 g, 450 µl of the upper phase was transferred into 400 µl isopropanol (Reanal), followed by incubation at -20°C for one hour. Samples were centrifuged for 15 min at 4°C, 16.000 g, followed by washing the nucleic acid pellet in 70% ethanol (Reanal). The pellet was finally eluted in 35µl of nuclease-free water. The same protocol was used for PUUV RNA extraction, with the exception that the source was supernatant of Vero E6 tissue culture inoculated with PUUV.

In the case of clinical samples, native human blood was centrifuged at 5000 x g, for 10 min at room temperature, followed by RNA extraction using the QIAamp Viral RNA Mini Kit, according to the manufacturers' recommendations.

4.3 Production of the recombinant antigens

4.3.1 PUUV nucleocapsid protein

The source of DOBV nucleocapsid protein was the prototype Sotkamo strain, isolated in the isle of Puumala from a bank vole in Sotkamo, Finland and was adapted to Vero E6 cells (Schmaljohn et al., 1985). The PCR reaction mix had a final volume of 25µl containing 1x AMV puffer (10 mM Tris, 50 mM KCl, 3 mM $MgCl_2$, pH 8,3), 0,4 mM dNTP mix (Promega), 2U AMV-RT (Promega), 4U nuclease inhibitor (Promega), 0,05 µM dithiothreitol, 50 pmol primer. 5 µl of RNA was used as template. The reverse transcription reaction was carried out at 42°C for 60 min.

PCR was carried out in a final volume of 50µl, containing 1x GoTaq® Flexi Buffer, 4 mM $MgCl_2$, 0,8 mM dNTP mix, 50 pmol 5'forward primer, 50 pmol 3'reverse primer 1,25U GoTaq® polimerase (Promega), 5 µl cDNS. The cycling conditions were: 40 cycles of denaturation at 95°C for 5 min, annealing at 55°C for 45 s and extension at 72°C for 1 min.

4.3.2 DOBV nucleocapsid protein

For DOBV nucleocapsid antigen, the N gene was amplified from a naturally infected yellow-necked mouse. The production of recombinant nucleocapsid protein was identical to the production of DOBV NP, discussed in detail above (Németh et al. 2011).

4.3.3 Cloning and transformation

PCR amplicons of the nucleocapsid gene were cloned into pGEM-T vector system (Promega) followed by cloning the fragment pET28a (Novagen) plasmid. 2µg of purified both vector types were used for dual restriction endonuclease digestion, using 2U of NdeI and XhoI (New England Biolabs) endonucleases and adequate enzyme buffer (20 mM Tris-acetate, 50 mM potassium-acetate, 10 mM magnesium-acetate, 1 mM DTT, pH 7,9). Digestion reactions were incubated for 2 h at 37°C. Digested inserts were then separated by agarose gel electrophoresis, lastly, the target DNA fragment was purified from gel (QIAquick Gel Extraction Kit, Qiagen).

The ligation was carried out at 15°C overnight (18 h), using 50ng vector plasmid, 30ng insert DNA, enzyme-compatible ligase buffer (30 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, pH 7,8), and 1 U T4 DNS ligase (Promega).

The construct „pET28a(+)–PUUV" was first transformed into 50 µl E. coli DH5 α competent cells (Promega), with the addition of 5 µl construct DNA. After incubating on ice for 20 min, the reaction mix was heat shocked at 42°C for 50 sec, followed by prompt replacement of samples into ice. After a minimum of 2 min cooling, 450µl of sterile Luria Broth (LB) was added, and transformed cells were incubated for 1 h at 37°C in water bath shaker at 200 rpm. 100 µl of cells were then plated on LB agar plates, containing 30µg/ml kanamycin, as selective agent. Plates were incubated at 37°C. Colony formation was checked after a minimum of 18 h incubation. Colony PCR was carried out picking 10 individual colonies to check for positive transformants.

After colony PCR, plasmid-containing colonies were propagated in LB broth with 30µg/ml kanamycin, followed by isolation of plasmids from cell pellet using Wizard® Plus SV Miniprep Kit, (Promega), according to the manufacturer's instructions. Purified plasmids were then transformed into BL21 Rosetta (DE3)pLysS (Novagen) competent cells, by the method described above.

4.3.4 PUUV nucleocapsid protein expression

Competent cells containing the recombinant pET28a plasmid were cultured in LB medium containing 30μg/ml kanamycin and 35 μg/ml chloramphenicol at 37°C, in a rotary shaker 200 rpm, until an OD_{600} of 0.8-1.0 (log phase. Cells were then induced with 1.0 mM IPTG, after incubation at 15°C for 20 hours, followed by centrifugation at 4°C, at 3000 g for 20 minutes. Cell pellet was stored until further processing at -20°C.

4.3.5 Protein purification and refolding

Pellet of the induced bacterial cells were resuspended in Bacterial Protein Extraction Reagent® (B-PER; Thermo Scientific), according to the manufacturers' recommendations. In short, 4 ml of B-PER reagent, 8 μl lysozyme (50 mg/ml), and 8μl DNase I enzyme (2500U/ml) was added to 1g of wet weight pellet, followed by incubation at room temperature for 20 min. Denatured cell debris was pelleted by centrifugation at 4°C for 30 min at 20.000 g, to separate dissolved proteins from inclusion bodies containing the expressed protein of interest. Inclusion bodies were lysed under denaturing conditions using lysis buffer containing 6 M guanidine-HCl, 0.1 M NaH_2PO_4, 0,01 M Tris HCl, pH = 8). Cell debris was removed from the lysate with centrifugation at 10.000 g for 1 min.

Recombinant proteins were purified also under denaturing conditions, utilizing HIS Select® HF Nickel Affinity Gel (Sigma Aldrich) column. Viral nucleocapsid proteins were bound to the affinity gel at 4°C, flowing the lysate three time through the column. Elution was carried out by a descending pH elution agent (8M Urea, 0.1M NaH_2PO_4, 0.01 M Tris), decreasing pH by 0,5 from pH 8.0 to pH 4.0. After elution, both the flow-through and elution fractions were separated by SDS-polyacrylamide gel electrophoresis (Mini-PROTEAN® TGX; Bio-Rad) and visualized by Coomassie blue staining (Brillant Blue R, Sigma).

Proteins were renatured via dialysis (dialysis membrane: 12.4 kDa; Sigma). During the dialysis process, urea concentration of the original elution buffer was gradually decreased by applying increasingly diluted dialysis buffer (6 M urea, 20 mM HEPES, pH = 7.9; 300 mM KCl, 5 mM $MgCl_2$, 10% glycerol, 0.5 mM PMSF).

Exact protein size and concentration was determined using Agilent High Sensitivity Protein 250 Kit for the Agilent 2100 Bioanalyzer Instrument (Agilent Technologies), according to the manufacturers' instructions.

4.4 Combined DOBV-PUUV specific ELISA

For ELISA and WB analysis, we applied the recombinant DOBV and PUUV antigens, both produced in an Escherichia coli bacterial expression system as described above. Microtiter plates (Maxisorp Nunc-Immuno Plate, Nunc) were coated with a combination of DOBV and PUUV antigens, followed by incubation for two hours at 37ºC. Excess antigens were removed by washing with phosphate-buffered saline (PBS) (pH 7.5), containing 0.05% Tween-20 (Sigma) (PBS-T). Microtiter plates were blocked for two hours at 37°C, using PBS containing 5% non-fat dry milk (Blotting-Grade Blocker, BIO-RAD) and 5% sucrose (Merck). Volunteers' sera were used in 1:100 dilution in PBS containing 2.5% non-fat dry milk. Serum samples of patients previously verified for DOBV and PUUV infections, respectively, were used as positive control. After incubation for one hour at 37°C, microtiter plates were washed with PBS-T. Horseradish peroxidase-conjugated rabbit anti-human IgG and IgM (DakoCytomation) were used as secondary antibodies, diluted to 1:4000 in PBS-T. Plates were incubated at 37°C for one hour, followed by washing again with PBS-T. Color development was allowed using 100 µl of 3,3',5,5'-tetreamethylbenzidine substrate (BD OptEIA), incubating at room temperature in the dark for 15 minutes. The reaction was stopped by the addition of 100 µl of 2M sulfuric acid, and optical density was measured on a microplate spectrophotometer (Thermo Electron Corporation) at 450 nm. The cut-off (CO) value was calculated as described previously (Dobly et al., 2012). Briefly: the average optical density (OD) of negative control sera plus 3 times their standard deviation. We compared series by calculating the ratio: observed OD/CO. A sample was considered positive if this ratio was at least 1.5. Ratios between 1.0 and 1.5 were considered ''grey zone'' values, while samples with ratio lower than 1 were considered to be negative.

4.5 Western blot assay

All samples considered as positive (with high or "grey zone" OD values) were confirmed by immunoblot analysis, against both DOBV and PUUV nucleocapsid

proteins. Nucleocapsid proteins of DOBV and PUUV were loaded into the wells of Mini-PROTEAN® Precast Gels (BIO-RAD). Polyacrilamide gel electrophoresis was carried out at 180V for 30 mins. After electrophoresis, proteins were transferred to 0.45μm pore size nitrocellulose membranes (BIO-RAD), using Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell (BIO-RAD), at 170 mA for 30 minutes. After blocking for 30 min with 5% non-fat dry milk (Blotting-Grade Blocker, BIO-RAD), sera were applied in 1:100 dilution using tris-buffered saline (TBS) (pH 7.5), containing 0.1% bovine serum albumin and 0.05% Tween-20 (Sigma). Horseradish peroxidase-conjugated rabbit anti-human IgG (1:2000 and rabbit anti-human IgM (1:1000 DakoCytomation) was used as secondary antibody. All incubation steps were executed at room temperature for 1 hour. Three consecutive 10 min washing steps were implemented using 1x TBS-Tween-20 (pH 7.5). Development was carried out using diamino-benzidine in 50 mM Tris (pH 7.5), containing 0.3% Nickel(II) chloride (Sigma).

4.6 ELISA and Western blot validation

Specificity and sensitivity were determined by comparing our WB analysis results to those of a commercially available ELISA test, NovaLisa (NovaTec), specific for DOBV, Hantaan, PUUV and Seoul viruses. For the comparison, we obtained 10 samples positive for DOBV and 10 samples for PUUV, while 40 negative sera from healthy persons.

4.7 TaqMan Resverse-transcriptase PCR

RNA from 200 μl sera was extracted by QIAamp Viral RNA Mini Kit (Qiagen), following the supplied manual. Primers for DOBV and PUUV one-step TaqMan RT-PCR were selected as described previously by Weidmann et al. (2005) and Garin et al. (2001). We performed the amplifications on a LineGene 9600 instrument (Bioer), using QIAGEN OneStep RT PCR Kit (Qiagen) and a temperature profile of RT at 42 °C for 30 min, activation at 95 °C for 5 min, and 50 cycles of PCR at 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 20 s. For positive control, we used DOBV and PUUV RNA extracted from infected rodents that were captured locally, in the Southern Transdanubian region of Hungary.

4.8 Sequencing of TaqMan RT-PCR products

Positive PCR products were loaded into 2.5% agarose gel, and fragments were isolated using QIAquick Gel Extraction Kit (Qiagen), according to the manufacturer's instructions. Sequencing PCR was carried out using Big Dye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems), with approximately 20 ng of template, followed by 25 cycles of PCR at 95 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. Amplified DNA was precipitated with absolute alcohol and suspended in Hi-Di™ formamide, (Applied Biosystems). ABI prism 310 Genetic Analyzer was used for fragment sequencing.

4.9 Phylogenetic analyses

Nucleic acid sequences of the hantavirus strains originating from patients were compared to reference strains uploaded in GenBank, and identification was established based upon the greatest identity. Sequences were edited and aligned using GeneDoc 3.2 and ClustalX 2.0 software, respectively. The tree was constructed with MEGA v5.0 software using the maximum likelihood method, based on the general time- reversible model (GTR+ G + I). Number of bootstraps for simulations was 1000.

4.10 Statistical analyses

For the comparing seroprevalence results between different main regions of Hungary, data were analyzed by Chi-squared test. Difference of $p \leq 0.05$ was considered as statistically significant for each analysis. Sample randomization was carried out using the built-in randomizer tool of Microsoft Excel 2010.

5. Results

5.1 PUUV recombinant nucleocapsid protein

As the basis of our serological screenings and western blot confirmations, we decided to utilize whole nucleocapsid proteins of DOBV and PUUV. Since DOBV production was carried out in frames of a previous work (Németh et al., 2011), hereby we only introduce detailed protein products for the PUUV NP only.

Although protein expression in a bacterial system is relatively easy and cost-effective, it has its shortcomings regarding the quality of the protein of interest. At first, large amounts of foreign proteins produced in *E. coli* do not remain in soluble form, but is rather deposited in crystal-like inclusion bodies. This fact has the disadvantage of having to utilize harsh protein purification techniques, as inclusion bodies have to be dissolved under strong denaturing conditions. On the other hand, inclusion bodies only contained our recombinant protein of interest, furthermore, proteases could not affect the expressed PUUV NP protein. Using 6M guanidine-HCl as a denaturing agent, the protein lysate was bound to Ni-NTA resin and eluted under continued denaturing conditions using 8M Urea of decreasing pH fractions. The whole PUUV NP appeared at pH = 5, with the most efficient elution at pH = 4.5, and finally also appearing at pH = 4.0 (Figure 7, A). In downstream ELISA and WB tests, NP was used in renatured form; hence all pH fractions of 5, 4.5 and 4 containing most of the protein were dialyzed. This enabled the use of a protein conformation more close to the natural form at the cost of losing some of the initial concentration in urea.

In the next step, protein expression products were characterized. Polyacrylamide gel electrophoresis as well as microfluidic-based analyses revealed 55 and 59 kDa-sized recombinant nucleocapsid proteins for DOBV and PUUV, respectively. Figure 7 B displays a 10-fold serial dilution of PUUV NP, showing weak protein signals with lower than 59 kDa size in the more concentrated dilutions. These were considered as being incomplete proteins produced during bacterial expression. Concentrations were measured as 400 ng/µl for DOBV and 2000 ng/µl for PUUV.

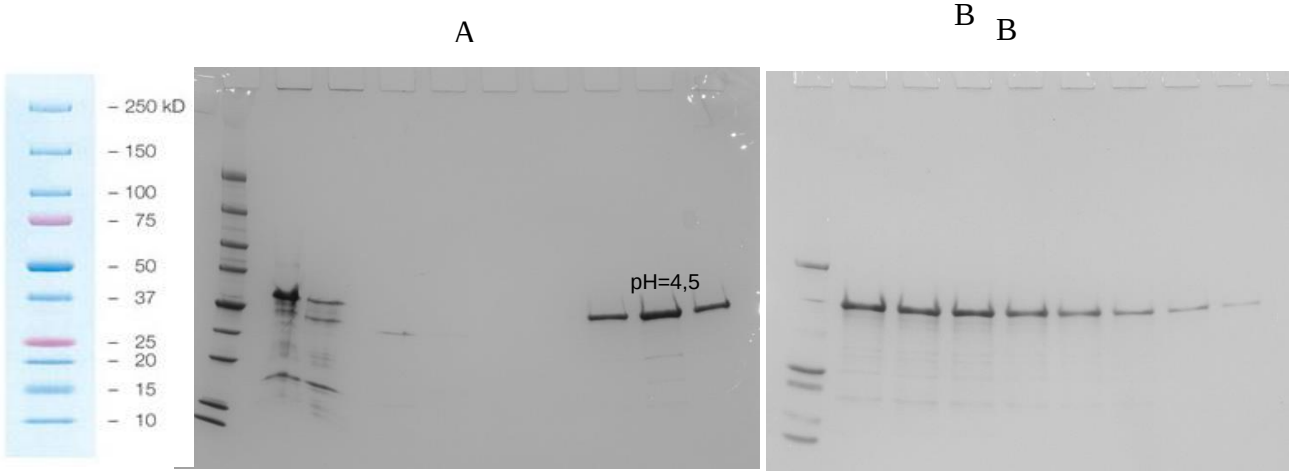

Figure 7. *PAGE image of purified PUUV nucleocapsid proteins.* ***A:*** *PAGE displaying the Ni-affinity purification result with a descending elution of urea. PUUV NP protein elutes most well at pH = 4.5.* ***B:*** *PAGE image of PUUV eluted protein in a serial dilution.*

5.2 ELISA and Western blot validation

Recombinant viral PUUV NP was at first tested by WB to determine activity. Anti-6xHis antibody specific for the His-tag at the N-terminal of the recombinant PUUV NP, as well as PUUV-positive human sera were used. Both proteins produced strong signal that indicated the proteins' active state. The 6xHis signal pattern does not exist in nature; hence its positive reaction proves the presence of the recombinant PUUV NP beyond doubt. Sera of healthy hantavirus-negative persons were used as negative control.

Next step was determining specificity and sensitivity values for both WB and ELISA tests. The same sample set, consisting of 40 negative, 10 DOBV-positive and 10 PUUV-positive samples, was tested in parallel with ELISA, WB assay and a commercially available ELISA kit (Hantavirus IgG, and IgM DxSelectTM ELISA kit, Focus Diagnostics) which contains DOBV, PUUV, HTNV and SEOV viral antigens as detecting agents.

Sensitivity was determined by dividing the number of true positives by the sum of true positives and false negatives, expressed in percentage. Specificity was calculated by dividing true negatives by the sum of true negatives and false positives (also expressed in percentage).

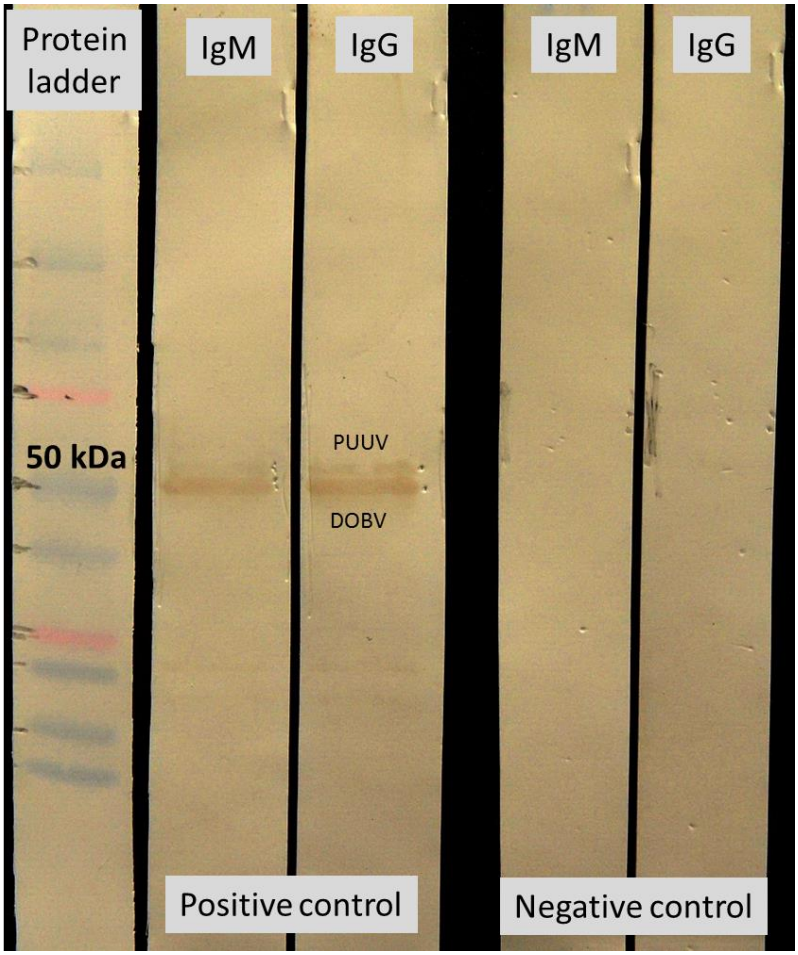

***Figure 8**. Representative immunoblot. DOBV and PUUV viral proteins are loaded into the same well; after immunoblotting, PUUV protein is found slightly above DOBV due to size characteristics, i.e. 55kDa and 59 kDa for DOBV and PUUV, respectively.*

Testing DOBV-positive samples by ELISA, all 10 true positive samples tested positive with our in-house ELISA assay, while resulting two false positives compared to the Focus Diagnostics kit. Assaying PUUV-positive samples, only 9 out of 10 true positives were found positive also by our in-house ELISA compared to the Focus Diagnostics kit, again resulting in two false positives. Hence, the sensitivity of ELISA for DOBV and PUUV was 100% and 90.9%, respectively, while the assay had a specificity of 95.2% for both viral antigens.

Regarding Western blotting, all 10 true positive DOBV and PUUV samples were positive by both the WB and the Focus Diagnostics kit, meaning a 100% sensitivity for both viral antigens. Finally, with zero false positives against DOBV and one false positive against PUUV, our WB assay resulted in a specificity of 100% and 97.5% for DOBV and PUUV, respectively.

5.3 Hantavirus incidence among patients hospitalized with AKI in the university hospital of Pécs

From January 2011 to December 2015, based on having fever, high leukocyte count, thrombocytopenia, elevated serum creatinine and/or transaminase levels, as well as urinary abnormalities, 94 persons were declared suspicious for hantavirus infection; therefore, they were enrolled into our clinical study. All patient samples were tested and found to be negative for leptospirosis. Out of the 94 patients, 69 (73.4%) were male and 25 (26.6%) were female. Their median age was 52 years, ranging from 9 to 77 years, all of them being inhabitants of the Transdanubian region of Hungary, that can be considered endemic for hantaviruses. The seasonality of hantavirus infections usually have a spring and autumn peak, fluctuations usually following rodent population sizes. During our investigation period between 2011 and 2015, patients with acute infections were hospitalized between June and December months with one case each month, an exception being October with two cases.

Based on the serologic assays, altogether 9 patients (9.6%) tested positive with WB analyses for hantaviruses. Seven persons (7.4%) had concurrent positive IgM and IgG findings they were considered having acute infections, while 2 persons (2.1%) tested positive for anti-hantaviral IgG only. For the latter two patients, hantavirus infection was not accepted as the cause of disease. In total 85 patients (90.4%) tested negative for hantavirus infection. Collected serum samples were also subjected in parallel to one-step TaqMan RT-PCR. None of the serologically negative samples were positive with molecular detection, while only in a single acute HFRS case (Patient 2), the viral etiology was proven by identifying hantavirus RNA in the sample. In the latter case, the phylogenetic analysis of the short 106 bp TaqMan RT-PCR fragment revealed an unequivocal DOBV infection.

Although human IgM and IgG usually display cross-reaction between PUUV and DOBV antigens, in an acute case, a WB test can be positive against PUUV and DOBV simultaneously. However, the colour development against the specific viral antigen is always visibly more prominent. According to this phenomenon, all seven acute-case patients had DOBV infection. Notable clinical symptoms and indicative laboratory parameters along with the highest values are summarized in Table 1.

Amid clinical signs, gastrointestinal symptoms are dominating, while haemorrhagic manifestations (as a prognostic sign for a severe disease course) were

only recorded in one case, gastroscopy for Patient 1 revealed haemorrhagic gastritis. High-grade fever is frequent, albeit not necessarily occurring even in DOBV infection cases. Headache and dizziness was apparent for Patients 1, 4 and 6. Patient 1 had a rapid weight increase of 6 kg during the first 9 days of her hospitalization. Patient 2 experienced the swelling of limbs. Both of the latter two clinical signs are the result of significant AKI. Patient 1 also complained of blurred vision, an indicator of a more severe hantavirus infection.

Among laboratory findings, low platelet count is the most indicative feature accompanied by elevated serum creatinine concentration. In our results, all seven acute-case persons with hospital documentation had thrombocytopenia and AKI (high serum creatinine concentration) concurrently. Elevations in leukocyte count (as a sign of infection) show greatly varying values. Elevations of serum transaminases were usually mild or non-existent.

Urinary abnormalities are almost always evident; in fact, in accordance with high serum creatinine concentration, it is typically the appearance of oliguria/anuria due to which patients visit the hospital or the emergency admissions unit. After blood testing during the first days of hospital stay, associated laboratory parameters raise the suspicion of hantavirus infection. In our investigation, all patients had significantly decreased urine output accompanied by haematuria and/or proteinuria. Finally, no patient required haemodialysis treatment, indicating a relatively favourable disease course.

Among the seven acute infections, in only case personal activity before disease could be determined as a supportive factor for hantaviral infection, Patient 2 is a frequent hiker; she visited local forests also just before falling ill.

Table 1. *Laboratory parameters of acute-case patients who tested positive for hantavirus.*

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**
Virus detection							
Western blot IgM results	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**
Western blot	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**

IgG results							
Clinical findings	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**
Headache	Yes	No	No	Yes	No	Yes	No
Myalgia	No	No	No	No	No	No	No
Gastrointestinal symptoms	Yes	Yes	No	Yes	Yes	Yes	Yes
Haemorrhagic manifestations	Yes	No	No	No	No	No	No
Highest body temperature (36 – 36.9 °C)	39.9	38.2	40	39	N/A	37	39
Hospital (days)	15	14	14	11	7	10	9
Laboratory findings (normal range)	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**
Highest leukocyte count (4-10 G/l)	14.1	11.7	8,2	7,88	15.3	9	8.44
Lowest haemoglobin concentration (120-170 g/l)	112	135	130	124	129	124	129
Lowest platelet count (142-424 G/l)	24	30	54	131	179	355	153
Highest serum creatinine concentration (60-120 µmol/l)	267	488	352	681	531	378	505

Highest AST concentration (<44 IU/l)	114	48	116	27	29	33	41
Highest ALT concentration (<50 IU/l)	127	11	108	23	20	54	78
Highest GGT concentration (<70 IU/l)	179	23	27	20	26	84	59
Urinary abnormalities	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**
Oliguria/anuria	Yes	Yes	No	Yes	Yes	Yes	Yes
Haematuria (≥5cells/FV**)	Yes	Yes	No	Yes	Yes	Yes	No
Proteinuria (≥1.5 g/day)	Yes	Yes	No	Yes	Yes	Yes	Yes
Haemodialysis treatment	No	No	No	No	No	No	No

5.4 Seroprevalence among hunters and forestry workers

In total, 835 blood samples were collected from healthy volunteer forestry workers, personnel of 106 silvicultures in 9 Hungarian counties. The 750 males (range: 18–70) and 85 females (range: 23–59) had an average age of 45 years, with 25 years (range: 0.1–51) of field work on average. The initial screening of the 835 serum samples in IgG ELISAs using mixed PUUV and DOBV antigens demonstrated a total of 45 (5.4%) reactive and equivocal sera. Serological reactivity for 38 (4.6%) of the 45 ELISA- reactive sera was confirmed in Western blot tests, the resulting 38 sera were regarded as seropositive.

A map displaying seroprevalence data was constructed, by dividing the country area to 4 main regions, which correspond to the field work area of donors. The main areas are the following: Northern Transdanubian region (I), Southern

Transdanubian region (II), the Danube-Tisza Plateau (III) and the North Hungarian Mountains (IV) (Figure 9).

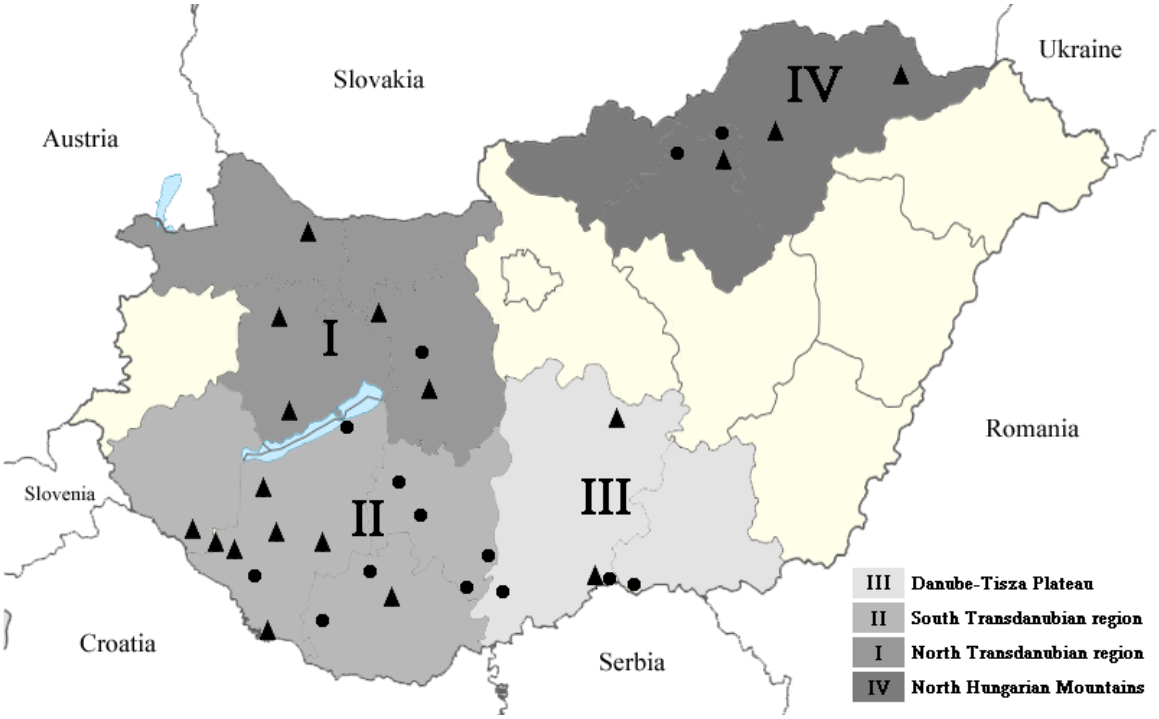

Figure 9. *Location of blood sampling sites and regions in Hungary. I. – Northern Transdanubian region, II – Southern Transdanubian region, III – Danube-Tisza Plateau, IV – North Hungarian Mountains. Black circles (●) represent sampling sites with no positive sera, black triangles (▲) represent sampling sites with positive sera.*

The totally collected and tested 835 sera were distributed by country regions as follows: 248 samples from 6 sampling sites from region I; 321 samples from 16 sampling sites in region II; 107 samples from 5 sampling sites in region III;, whereas 159 samples from 5 sampling sites in region IV. Total seroprevalence was 4.6%, while region IV had the local highest value of 8.2%. Table 2 represents the raw data we got from the total number of samples tested.

Table 2. *Total sera collected by country regions and regional seropositivity, raw data*.

Country region	**No of sampling sites**	**No of samples**	**No of positives**	**Seroprevalence (%)**
Danube-Tisza Plateau (III.)	5	107	2	1.9
Southern Transdanubian region (II.)	16	321	10	3.1
Northern Transdanubian region (I.)	6	248	13	5.2
North Hungarian Mountains (IV.)	5	159	13	8.2
Total	**32**	**835**	**38**	**4.6**

However, since there are relatively large differences in sample numbers between the particular country regions, a statistical solution was needed to bring different sample amounts to a comparable level. Thus, we chose to compare 100 samples from each region, since this is close to lowest sample number per region (III) From all regions, the 100 samples were selected randomly. The idea of stratified random sampling was rejected due to the fact that in case of region III, only 14 sera would be enrolled, which we considered too low to provide reliable information. Normalized data are represented in Table 3.

Table 3. *Total sera collected by country regions and regional seropositivity corrected by randomized sampling*

Country region	**No. of samples**	**RND samples chosen**	**No. of positives**	**Seroprevalence (%)**
Danube-Tisza Plateau (III.)	107	100	2	2.0
Southern Transdanubian region (II.)	321	100	3	3.0
Northern Transdanubian region (I.)	248	100	7	7.0
North Hungarian Mountains (IV.)	159	100	10	10.0
Total	**835**	**400**	**22**	**5.5**

.

After data normalization, total seroprevalence increased to 5.5 % while seroprevalence remained the highest in region IV, with 10.0%, followed by region I, 7%, whereas regions II and III had lower values of 3% and 2%, respectively.

6. Discussion

6.1 Hantavirus incidence among patients hospitalized with AKI in the university hospital of Pécs

In hantavirus diagnostics, immunoassay techniques, such as ELISA, WB and IFA are most frequently used as routine methods. The downside is that commercially available assays are relatively expensive, and they are only cost-effective when testing a large number of samples. Among immunological methods, WB assay has the advantages of high sensitivity and also specificity, moreover, samples can be tested one at a time without being wasteful. Real-time RT-PCR, while being one of the most up-to-date tools, has a certain drawback when testing for hantaviruses: viral titre in blood or other body fluids (urine, saliva) is usually low, and viremia lasts for a relatively short period, thus a negative RT-PCR finding does not undoubtedly provide a negative diagnosis. In our study, viral RNA could only be detected in a single case out of eight acute infections.

Although hantavirus serotyping is rarely successful with ELISA or WB analyses, we used a mix of recombinant antigens of the two locally common pathogenic hantavirus types (DOBV and PUUV). In all positive cases independently of IgG or IgM positivity, we detected strong positive signal with both antigen, which confirm the cross-reactivity between different hantavirus species in immunological assays. On the other hand, the latter signal intensity was always more prominent against the specific viral antigen which really caused the given infection (in our study, DOBV). A reason behind cross-reactions might be the utilization of whole nucleocapsid proteins, which contain both genotype specific and type common immunoreactive epitopes. Serum of "Patient-2" reacted also with PUUV nucleocapsid protein, while her infection was confirmed as DOBV at the nucleotide sequence level. Considering the above mentioned, it is clearly demonstrated that our WB assay using bacterial expressed recombinant antigen can be used for detection of hantavirus infection, but due to cross-reactivity, the assay is not directly suitable for genotyping. However, with specificity and sensitivity values of 97.5% (PUUV) and 100% (DOBV), the test is highly acceptable for clinical diagnostic purposes, where the actual infective genotype is not a crucial point from the aspect of treatment. Treatment

in case of a hantavirus infection is always supportive, but by excluding bacteria or fungi as other possible infective agents, physicians can avoid the unnecessary use of antibiotics, thereby circumventing numerous side-effects.

The follow-up sera of Patient-2 revealed that IgM antibodies against hantavirus were present in blood even after ten months. Usually, hantavirus-specific IgM disappears two to three months after the onset of symptoms (Tai et al., 2005). However, persistent IgM antibodies against recombinant hantaviral NPs have been reported previously in DOBV-infected patients for as long as two to three years after hospitalization, although with very low titres (Meisel et al., 2006).

In all seven cases, which were assumed as acute infections, fever, AKI, thrombocytopenia and elevated transaminase levels were apparent as characteristic findings of hantavirus infection, described previously in several papers (Earle, 1954, Krautkrämer et al., 2013 Vaheri et al. 2013). In one case, haemorrhagic manifestation also occurred. We were able to detect viral nucleic acid in a single case. RT-PCR was negative in the rest of the cases. Personal activity of one patient could be considered probably as a supportive circumstance for DOBV infection.

Leading clinical manifestations were fever, accompanied by gastrointestinal pain and vomiting. Urinary deviations (oliguria and/or haematuria) were apparent in six of the seven acute cases. Haemorrhagic manifestation was observed only for Patient 1 (haemorrhagic gastritis).

The main laboratory indicators are thrombocytopenia and elevated serum creatinine. The former is the result of cytokine-mediated endothelial damage and the increase of capillary permeability, while elevated serum creatinine is the indicator of AKI.

6.1.1 Comparison of clinical cases with the surrounding region

In the Balkans, also DOBV and PUUV are the hantavirus types associated with clinical manifestations. HFRS cases are either sporadic or are associated with major outbreaks, influenced by the abundance of rodent host populations. Although disease incidence is relatively low, DOBV – as seen in the current study – is responsible for the more severe cases, resulting in a mortality rate of up to 12% (Avšič Županc et al., 1999). Asymptomatic infections in humans were caused at significantly higher percentage by PUUV (69.8%) than by DOBV (17.5%) (Hukic et al., 2011). The most common clinical findings are fever, chills, malaise, myalgia, back and abdominal

pain, headache, vomiting, diarrhoea, blurred vision, and oliguria followed by polyuria (Avšič Županc et al., 1999; Markotić et al., 2002b). All the latter are completely in accordance with our experiences. Blurred vision, acute renal failure, diarrhoea and melena are significantly more often registered in patients with DOBV infection in Croatian patients (Markotić et al., 2002b). However, in Slovenian patients, blurred vision was significantly more often recorded in patients with PUUV infection (Avšič Županc et al., 1999). In the current study, only Patient 1 complained of blurred vision. This was also the only case where viral RNA was detected, indicating a more severe infection associated with DOBV genotype and a higher viral load.

In a 2013 study, Korva et al. had the interesting finding that viremia lasts longer than previously assumed. Patients infected with DOBV or PUUV have a viremia lasting on average 30 days or 16 days, respectively. DOBV infected patients were found to have a higher viral load than the PUUV infected patients. Furthermore, both DOBV and PUUV infected patients had IgM at the time of hospital admission, but there was a difference in IgG antibody dynamics, i.e. only a minority of DOBV infected patients had IgG antibodies (Korva et al., 2013b). The latter findings are in sharp contrast with our experiences. Firstly, we expressed strong emphasis on the difficulty of molecular detection of hantaviruses from patients: despite that all samples originated within 16 days POS, hantaviral RNA was only detected on a single occasion. Secondly, in our immunoblot tests, DOBV-specific IgG was clearly present in all acute cases. Like in other parts of the Eurasian continent, renal disorder is a leading symptom of HFRS in the Balkans. Elevated levels of urea and creatinine are helpful in early HFRS diagnosis. Oliguria is common in at least one third of patients; anuria and acute renal failure require temporary haemodialysis (Avšič Županc et al., 1999; Markotić et al.,2002b). In comparison, all patients in the current study had elevated levels of serum creatinine, six out seven persons had oliguria, but none of them required haemodialysis treatment.

Due to the high diversity of clinical signs and symptoms, differential diagnosis is can initially be complicated: acute renal failure of another etiology, acute febrile urinary tract infection, tubulointerstitial nephritis of other etiology, acute and chronic glomerulonephritis, acute abdomen, including appendicitis, haemorrhagic scarlatina, haemolytic uremic syndrome (HUS), thrombocytopenic thrombotic purpura (TTP), acute respiratory infections, sepsis. Among all the above listed syndromes, acute

appendicitis, mimicking HFRS was found earlier by our research group (Jakab et al., 2011). As in Hungary, leptospirosis is in the first place in differential diagnosis of HFRS in the Balkans. Both causative agents have rodent reservoirs, both are endemic in South-East Europe and have mostly identical symptoms and clinical laboratory findings at the onset of disease. Patients with leptospirosis usually have calf pain and higher levels of ALT, AST and especially total bilirubin (Markotić et al., 2002a; Sion et al., 2002).

Co-infections of host animals with Leptospira and hantaviruses were studied in Croatia, where 16% of the 44 totally tested rodents (yellow-necked mice and bank voles) harboured both pathogens, while 3 out of 28 (11%) yellow necked mice were also infected with Babesia, besides the former two pathogens (Tadin et al., 2012). In Hungary, no study tested rodents in parallel for hantaviruses and Leptospira; however, in a single human case in the study region verified co-infection was described. The patient had haemorrhagic fever with mild, transient renal insufficiency and liver damage (Nemes and Péterfi, 2000).

Considering strictly clinical studies, data are available from some Balkan countries. In Albania, a study performed between 2003 and 2006 describes 11.7% of the patients with clinical suspicion of CCHF were confirmed as HFRS cases (Papa et al., 2008). In Bulgaria, approximately 2.8% of patients with acute undifferentiated febrile illness are due to hantavirus infections (Christova et al., 2013).

Summarizing our results, we demonstrated the pathogenic potential of hantaviruses in the area, with DOBV identified as an etiological agent in 7.4% of acute kidney injury cases, with suspected infection origin. The optimized WB method is suitable for the quick testing of a few or even a single sample, without sacrificing time and cost-effectiveness compared to commercially available ELISA kits, which are rather used for large-scale epidemiological screening or diagnostics in countries where a great number of acute cases appear annually. Hantaviruses are shown to be a fairly potential pathogen in the area. The antibody detection methods we developed will enable us to perform both large-scale surveillance and also single-case diagnostics that should help us to gain a clearer picture of the epidemiology and the ecology of hantavirus infections in Hungary.

6.2 Seroprevalence among hunters and forestry workers

The current study revealed a relatively high seropositive rate for hantaviruses among forestry workers in the North Hungarian Mountains (IV). The difference between areas IV and III was five-fold. In all areas, differences in seropositivity between sampling sites among the corresponding geographic region were observed. However, any specific sampling spot gathered workers from large areas (from up to 100 km radius of the sampling site), hence were regions created to make evaluation easier. Because the source of hantavirus infections in humans are rodents, this area-specific difference may reflect differences in the exposure to infected rodents. In Hungary, rodent hosts for DOBV are yellow-necked mice (*Apodemus flavicollis)* and field mice (*A. agrarius)*, while PUUV is carried by bank vole *(Myodes glareolus).* Although the habitat of field mice includes forests as well as agricultural areas, the remaining two host species, especially the yellow-necked mouse typically prefers large, montane woodlands for habitats. (Suchomel et al., 2007, Trubenovj and Miklós, 2007). These mature deciduous forests with sufficient plant diversity ensure an adequate food supply each year (Kryštufek, 1991; Vukićević-Radić et al., 2006, Avšič Županc et al., 2014). Our findings seem to correlate with the latter, as the highest seroprevalence values in this study were detected in the large mountain woodlands of regions IV and I. Statistical test results also corroborated that significant difference was only calculated when comparing region IV to regions II and III, the latter two being hilly and lowland regions. The considerably lower prevalence value in region III is probably related to the relatively small and open forest patches, where small mammal population gets mixed with various rodent and shrew species.

Studies similar to our survey have been carried out in Europe as well as in the Balkans to reveal hantavirus seroprevalence in forestry workers. In Slovakia, 2133 persons were involved in a screening for DOBV, PUUV and TULV hantaviruses. A 5.9% seroprevalence was detected in the eastern part of the country (Sibold et al., 1999). In Germany, a corresponding work was carried out in the north-eastern Brandenburg territory, revealing that among a total of 563 tested persons, 9% proved to be seropositive against DOBV, PUUV and TULV (Mertens et al., 2011). In Switzerland, the assaying of 1693 farmers, forestry workers, young soldiers and blood donors showed low seroprevalence rates with a maximum of 1.9%. The study area

was restricted to the Canton of St. Gallen (Schultze et al., 2007). There are two related studies from Poland. In the paper of Gut et al. 2007, panels of 86 sera from forest workers and 47 zoologists working with small mammals were evaluated for hantavirus specific IgG. PUVV-specific antibodies were detected in 14.9% (7/47) from zoologist's sera, while all sera of forestry workers were negative (Gut et al., 2007). On the other hand, Grygorczuk et al. in 2008 found that in the north-eastern part of Poland, anti-PUUV IgG from forest worker sera was detected in 5.7% (4/69), while HTNV-specific IgG was present in 2 persons in 2.9% (2/69) (Grygorczuk et al., 2008).

Regarding the Balkans, several additional studies dealt with hantavirus seroprevalence. In a 2010 study investigating the hantavirus-endemic region of Bosnia and Herzegovina, a total of 1331 persons were involved from endemic and non-endemic regions. The seroprevalence in the general population is 7.4% in the endemic region and 2.4% in the non-endemic region. A marked contrast in seroprevalence between risk population, i.e. former soldiers (16.1%) and the general population (6.2%) was revealed in the same area (Hukic M et al., 2010). A seroepidemiological investigation conducted in Croatia among 300 forestry workers and 260 individuals from the general population revealed that antibody prevalence ranged from 0% to 8.9%, with an overall rate of 1.6% (Borcić et al.,1991). In Greece, serological surveys processing a total of 2653 blood samples demonstrated an overall seroprevalence of 4%, ranging from 0% up to 14%, where higher percentage was observed among individuals with occupational risks (Antoniadis et al., 1987). In Slovenia, the general population showed hantavirus-specific antibody prevalence of 1.7% on average, but it varied from 0% to 14.3% when a group of woodworkers was investigated (Avšič Županc and Poljak,1994). In Romania, hantavirus diagnostic capabilities were established relatively late in 2008. Before, the disease was seemingly overlooked, with only 5 retrospectively confirmed HFRS cases (Maftei et al., 2012). Since 2008, an additional 17 HFRS cases were reported and all infections were confirmed as DOBV, most HFRS cases were connected with occupational hazards like shepherds or woodcutters (Heymanet al., 2011, Avšič Županc et al., 2014).

It is interesting to compare our results to a former large-scale study from Hungary. A national serosurvey was carried out in 2000, when 2257 citizens were tested for antibodies against hantavirus using IFAT, HDPA and ELISA. Results

indicated that about 10% of healthy people can be considered seropositive. Affected persons lived in all areas of the country (Ferenczi et al., 2003). Persons tested at the time were not restricted to the "risk population" discussed here, but were selected on a basis only to cover the majority of the country area and being older than 20 years. In the light of our results, the 4.5% disparity in total seroprevalence represents a marked discrepancy. This contradiction is further amplified by the general observation that in all studies that report relevant data, hantavirus seroprevalence in the suggested risk group is truly higher compared to the general population. Possible explanations include the differences in sensitivity and specificity of test results utilized in the present and the past Hungarian serosurvey. Our screening (ELISA) and confirmation (WB) methods were carefully optimized and were based on recombinant viral nucleocapsid antigens that were produced utilizing up-to-date molecular biological systems.

In conclusion, comparing Hungary to other European and Balkan countries with available data of hantavirus prevalence in any kind of risk population, we conclude that the overall seroprevalence of hantavirus infections is most similar to that of reported form Bosnia and Herzegovina and Slovakia, at least in terms of the overall values. Our results fit in the observed range of 1.7-16%, with our maximum result of 10% being roughly in the middle. The data shown in the studies from Poland also demonstrate comparable values, although there is a large difference in the number of tested samples. The authors from Greece, Slovakia and Switzerland screened considerably more persons, whereas the study from Croatia and Germany had the most comparable number of involved forestry workers. On the other hand, Germany experiences way higher number of annual hantavirus infections than Hungary and the countries directly surrounding it (Heyman et al. 2011, Krüger et al., 2013). Furthermore, TULV was included in the study from Germany and Slovakia showing that - at least in the federal state of Brandenburg -, more than 40% of the positive sera reacted exclusively with TULV. We would like to note however, that the pathogenic nature of TULV is still not confirmed. A single disease case with direct molecular evidence was reported in an immunocompromised person, (Zelená et al., 2013).

Given those whole nucleocapsid proteins that contain both type-specific and type-common epitopes, our laboratory tests could not differentiate DOBV and PUUV

infections. Similar shortcomings apply to several commonly used commercial and in-house laboratory assays developed for hantavirus serology. Therefore, more precise picture about the rates of infections caused by different pathogenic hantaviruses will required new methodological approaches.

In conclusion, based on the results of our comprehensive investigation in Hungary, we assume that hantavirus seroprevalence among forestry workers is similar to the Slovakia, moreover, hantavirus infection might deserve a greater attention concerning risk population in the future.

7. Summary

Hantaviruses are widespread infectious agents carried by various rodents and other small mammals. Their discovery and emergence in different localities throughout the world was always associated with wars, as it is wartime conditions when relatively large number of humans may get in close contact with disease-spreading animals. As hantavirus research widened and its diagnostic methods were refined, it became clear that in certain areas of the world, mainly in China, Russia, in the majority of Europe, and in several regions of the North and South Americas, the disease is considerably frequent and a high number of viral infectious cases with often severe course can be attributed to this viral agent. As in modern times people's habitat is increasingly expanding, overlaps in human and animal habitats provide even more opportunity for contracting the disease. Since there is only supportive treatment for hantavirus infection, research shifted towards the discovery of novel host species as well as towards the development of vaccines as preventive approaches is highly endemic areas.

Hungary is one of the European countries, where different hantavirus species are confirmedly circulating, and although the disease is relatively rare, the clinical consequences may worth the effort put into the assessment of current health risk, especially that related data are lacking since the beginning of the 21st century. With the above mentioned in mind, we set two main goals in the current work: the development of a relatively cheap and rapid method for diagnosing hantavirus infection, and the surveying of the country wide seroprevalence in the so called risk population, whose profession provides frequent contact with hantavirus-spreading animals. The risk population was determined as persons being hunters and forestry workers.

In Hungary, DOBV and PUUV are considered to be the main circulating hantavirus species that are also capable of causing illness in humans. Hence, we optimized an ELISA for the screening of a large number of volunteers from the risk group, and a WB method for confirmation of ELISA results, as well as a tool for single, acute-case hantavirus diagnostics. In both methods we used DOBV and PUUV NPs expressed in an *E. coli* system as the detecting viral antigen.

DOBV antigen was produced during a previous study, while PUUV NP was newly expressed in a similar *E. coli* system, and is the first main result of the current thesis. With both viral antigens at hand, the optimization of the ELISA resulted in a sensitivity of 100% and 90.9%, for DOBV and PUUV, respectively, while the assay had a specificity of 95.2% for both viral antigens. The WB method provided better results, with 100% sensitivity for both viral antigens, with a specificity of 100% and 97.5% for DOBV and PUUV, respectively. These values we considered suitable for the processing of samples.

The WB assay was tested as a potential diagnostic tool and in cooperation with the clinics of the University of Pécs, between 2011 and 2015, we assayed a total of 94 patient samples, that were considered suspicious for hantavirus infection by physicians. Among them we have found seven cases (7.4%), where human anti-hanta IgM and IgG tests were both positive, these cases were evaluated as acute infections and hantavirus was declared as the cause of disease. Clinical symptoms were moderate with impaired kidney function – termed acute kidney injury (AKI) –; whereas ruling indicative laboratory parameters were increased serum creatinine concentration, thrombocytopenia, proteinuria and haematuria. During this 5-year period, no one succumbed into the disease and all infections were determined to be caused by DOBV, which is known to elicit more severe disease courses compared to PUUV. We also found two persons, where only anti-hanta IgG was detected, these cases were viewed as past infections and hantavirus was not regarded as the causative agent at the time of testing during our study.

The collection of blood samples from volunteer hunters and forestry workers was carried out between 2012 and 2013. We tested a total of 835 sera that were distributed by four main country regions, corresponding to the work area of volunteers. Country regions were established as follows: Northern Transdanubian region (248 samples from 6 sampling sites), the Southern Transdanubian region (321 samples from 16 sampling sites); the Danube-Tisza Plateau (107 samples from 5 sampling sites) and finally the North Hungarian Mountains (159 samples from 5 sampling sites). Since sample numbers were highly varying between the respective regions, data were normalized and this way total seroprevalence was 5.5%, while local seroprevalence was the highest in the North Hungarian Mountains with a 10% seropositive rate followed by 7% in the Northern Transdanubian region. The Southern

Transdanubian region and the Danube-Tisza Plateau had significantly lower result values with 3% and 2%, respectively. These results correlate with the large coherent mountain forest regions in our country, where carrier animals have the largest populations. The 2-10% seroprevalence was in the range of results of similar European and Balkan studies (Bosnia and Herzegovina – 6%, Croatia – 1.7%, Greece – 4%; Germany – 9%, Poland – 2.9-14.9, Slovakia – 5.9%, Slovenia – 1.7%, Switzerland – 1.9%).

However, it is interesting and also may seem controversial that our maximum result of 10% hantavirus seroprevalence in the North Hungarian Mountains is equal to that found in a previous Hungarian large-scale study (Ferenczi et al., 2003), carried out more than a decade earlier, where a total of 10% seropositivity was determined. Over 2000 persons above 20 years of age were involved in the survey, who in contrast cannot be viewed a risk population, as they were selected randomly. These results either indicate that there is no difference in country-wide hantavirus seropositive rate between the risk population and the ordinary people, or there is difference between testing methods which wash away the expected result that members of the proposed risk group are more likely to get infected by hantaviruses (as indicated in all similar studies).

We consider the development of a WB method as a hantavirus diagnostics tool a success, as it could substitute commercially available hantavirus ELISA kits. The latter are relatively expensive, and are only cost-effective when testing a large number of samples simultaneously. In Hungary, hantavirus infection is relatively rare, so the time needed for the accumulation of enough samples for ELISA might negatively influence result return time in routine diagnostics.

The assessment of hantavirus seroprevalence in the proposed risk population – even despite some controversy when comparing to a previous home study with a different sample set – might serve as a gap-filling measure, as these kind of data were totally absent for Hungary.

8. Acknowledgement

I would like to thank the following people for all kinds of help and contribution to my work and hence the creation of the current thesis.

Firstly to Dr. Ferenc Jakab, my supervisor, who provided the laboratory background, the theoretical and practical direction, teaching and supporting for my work.

I thank Dr. Emőke Ferenczi for kindly providing us the Puumala virus Sotkamo strain supernatant, the source of RNA for PUUV nucleocapsid protein.

Furthermore, I thank Prof. Dr. István Wittmann, Dr. Judit Sebők and Dr. Zoltán Péterfi for providing clinical samples and additionally their medical knowledge that was crucial for writing the manuscripts forming the basis of the thesis.

My sincere thank goes to all volunteer hunters and forestry workers for their essential contribution by providing several hundred blood samples.

Thank goes to the members of Department of Medical Microbiology and Immunology, especially to Zsoltné Garamvölgyi and Csilla Wéber for their essential help in sample collection and management.

I would like to thank Prof. Dr. Detlev H. Krüger for kindly providing us the hantavirus-positive human sera as controls for the immunological experiments.

I thank Dr. Csaba Fekete for providing the Agilent 2100 bioanalyzer, used for the accurate size determination of the expressed viral proteins.

I would also like to thank all my colleagues, firstly Mónika Madai and Réka Pintér for their help in sample collection, and all the others, namely Viktória Németh, Renáta Dóró, Gábor Kemenesi, Bianka Dallos and Dr. Anna Kutas for their daily help and support in the laboratory.

A lot of thank goes to my wife for her support and encouragement throughout the whole working period, and beyond.

9. References

Aitichou M, Saleh SS, McElroy AK, Schmaljohn C, Ibrahim MS (2005): Identification of Dobrava, Hantaan, Seoul, and Puumala viruses by one-step real-time RT-PCR. *J Virol Methods;* 124(1-2):21-6.

Antoniadis A, Le Duc JW, Daniel-Alexiou S (1987): Clinical and epidemiological aspects of hemorrhagic fever with renal syndrome (HFRS) in Greece. *Eur J Epidemiol*; 3(3):295-301.

Avsic-Zupanc T, Xiao SY, Stojanovic R, Gligic A, van der Groen G, LeDuc JW (1992): Characterization of Dobrava virus: a Hantavirus from Slovenia, Yugoslavia. *J Med Virol;* 38(2):132-7.

Avšič-Županc T, Poljak M (1994): Hantavirus genomic variation and disease distribution

Zdrav Vestn; (63):25-27

Avšič Županc T, Korva M, Markotić A (2014). HFRS and hantaviruses in the Balkans/South-East Europe. *Virus Res* 2014 Jul 17;187:27-33.

Beaty BJ, Bishop DH (1988): Bunyavirus-vector interactions. *Virus Res;* 10:289–301.

Billecocq A, Coudrier D, Boué F, Combes B, Zeller H, Artois M, Bouloy M (2003):

Expression of the nucleoprotein of the Puumala virus from the recombinant Semliki Forest virus replicon: characterization and use as a potential diagnostic tool. *Clin Diagn Lab Immunol;* 10(4):658-63.

Bishop DH, Beaty, BJ (1988): Molecular and biochemical studies of the evolution, infection and transmission of insect bunyaviruses. *Philos Trans R Soc Lond Series B Biol Sci*; 321:463–483.

Borcić B, Turković B, Aleraj B, Tvrtković N (1991): [Hemorrhagic fever with renal syndrome in Croatia: incidence of infection in human and wild animal reservoirs]. [Article in Croatian] *Lijec Vjesn*; 113(9-10):320-3.

Brummer-Korvenkontio M, Vaheri A, Hovi T, von Bonsdorff CH, Vuorimies J, Manni T, Penttinen K, Oker-Blom N, Lähdevirta J (1980): Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. *J Infect Dis;* 141(2):131-4.

Buranda T, Wu Y, Perez D, Jett SD, BonduHawkins V, Ye C, Edwards B, Hall P, Larson RS, Lopez GP, Sklar LA, Hjelle B (2010): Recognition of decay accelerating factor and alpha(v)beta(3) by inactivated hantaviruses: Toward the development of high-throughput screening flow cytometry assays. *Anal Biochem;* 402(2):151-60.

Caramello P, Canta F, Bonino L, Moiraghi C, Navone F, Lipani F, Balbiano R, Caputo AM, Gai V (2002): Puumala virus pulmonary syndrome in a Romanian immigrant. *J Travel Med;* 9(6):326-9.

Clement J, Colson P, McKenna P (1994): Hantavirus pulmonary syndrome in New England and Europe. *N Engl J Med;* 331:545–6. [author reply 547–48].

Clement J, Maes P, Lagrou K, Van Ranst M, Lameire N (2012): A unifying hypothesis and a single name for a complex globally emerging infection: hantavirus disease. *Eur J Clin Microbiol Infect Dis;* 31(1):1-5.

Choi Y, Kwon YC, Kim SI, Park JM, Lee KH, Ahn BY (2008): A hantavirus causing hemorrhagic fever with renal syndrome requires gC1qR/p32 for efficient cell binding and infection. *Virology;* 381(2):178-83

Dearing MD, Dizney L (2010): Ecology of hantavirus in a changing world. Ann N Y *Acad Sci*; 1195:99-112.

Dobly A, Cochez C, Goossens E, De Bosschere H, Hansen P, Roels S, Heyman P (2012): Sero-epidemiological study of the presence of hantaviruses in domestic dogs and cats from Belgium. *Res Vet Sci;* 92(2):221-4.

Earle DP (1954): Symposium on epidemic haemorrhagic fever. *Am. J. 1\'led;* 16:619-709.

Elgh F, Lundkvist A, Alexeyev OA, Stenlund H, Avsic-Zupanc T, Hjelle B, Lee HW, Smith KJ, Vainionpää R, Wiger D, Wadell G, Juto P (1997): Serological diagnosis of hantavirus infections by an enzyme-linked immunosorbent assay based on detection of immunoglobulin G and M responses to recombinant nucleocapsid proteins of five viral serotypes. *J Clin Microbiol;* 35(5):1122-30.

Faludi G, Ferenczi E (1995): Serologically verified hantavirus infections in Hungary. *Acta Microbiol Immunol Hung;* 42(4):419-26.

Ferenczi E, Rácz G, Szekeres J, Balog K, Tóth E, Takács M, Csire M, Mezey I, Berencsi G, Faludi G (2003): New data for the public health importance of hantaviruses in Hungary. *Orv Hetil;* 144(109):467-74.

Ferenczi E, Rácz G, Faludi G, Czeglédi A. Mezei I, Berencsi G (2005): Natural Foci of Classical and Emerging Viral Zoonoses in Hungary. *Emerging biological threat;* 370;43-50.

Ferres M, Vial P, Marco C, Yanez L, Godoy P, Castillo C, Hjelle B, Delgado I, Lee SJ, Mertz GJ; Andes Virus Household Contacts Study Group (2007): Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in chile. *J Infect Dis;* 195(11):1563-71

Figueiredo LT, Moreli ML, Borges AA, Figueiredo GG, Souza RL, Aquino VH (2008): Expression of a hantavirus N protein and its efficacy as antigen in immune assays. *Braz J Med Biol Res;* 41(7):596-9.

Flick R, Bouloy M (2005): Rift Valley fever virus. *Curr Mol Med;* 5:827–834.

French GR, Foulke RS, Brand OA, Eddy GA, Lee HW, Lee PW (1981): Korean hemorrhagic fever: propagation of the etiologic agent in a cell line of human origin. *Science;* 211(4486):1046-8.

Gajdusek DC (1982): Muroid virus nephropathies and muroid viruses of the Hantaan virus group. *Scand J Infect Dis Suppl;* 36:96-108.

Garin D, Peyrefitte C, Crance JM, Le Faou A, Jouan A, Bouloy M. Highly sensitive Taqman PCR detection of Puumala hantavirus. *Microbes Infect* 2001;3:739–45.

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

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

Giebel LB, Zöller L, Bautz EK, Darai G (1990): Rapid detection of genomic variations in different strains of hantaviruses by polymerase chain reaction techniques and nucleotide sequence analysis. *Virus Res;* 16(2):127-36.

Gizzi M, Delaere B, Weynand B, Clement J, Maes P, Vergote V, Laenen L, Hjelle B, Verroken A, Dive A, Michaux I, Evrard P, Creytens D, Bulpa P (2013): Another case of "European hantavirus pulmonary syndrome" with severe lung, prior to kidney, involvement, and diagnosed by viral inclusions in lung macrophages. *Eur J Clin Microbiol Infect Dis;* 32(10):1341-5.

Gligic A, Dimkovic N, Xiao SY, Buckle GJ, Jovanovic D, Velimirovic D, Stojanovic R, Obradovic M, Diglisic G, Micic J, et al. (1992): Belgrade virus: a new hantavirus causing severe hemorrhagic fever with renal syndrome in Yugoslavia. *J Infect Dis;* 166(1):113-20.

Groen J, Bruijn JA, Gerding MN, Jordans JG, Moll van Charante AW, Osterhaus AD (1996): Hantavirus antigen detection in kidney biopsies from patients with nephropathia epidemica. *Clin Nephrol;* 46(6):379-83.

Grygorczuk S1, Pancewicz S, Zajkowska J, Kondrusik M, Swierzbińska R, Moniuszko A, Pawlak-Zalewska W (2008): [Detection of anti-hantavirus antibodies in forest workers in the north-east of Poland]. *Przegl Epidemiol;* 62(3):531-7.

Guhl S, Franke R, Schielke A, Johne R, Krüger DH, Babina M, Rang A (2010): Infection of in vivo differentiated human mast cells with hantaviruses. *J Gen Virol;* 91(5):1256-61.

Gut W, Siennicka J, Sadkowska-Todys M, Baumann A, Litwińska B (2007): [Hantavirus specific IgG antibodies in population of zoologists and forest workers]. *Przegl Epidemiol;* 61(3): 483-8.

Hautala T, Sironen T, Vapalahti O, Pääkkö E, Särkioja T, Salmela PI, Vaheri A, Plyusnin A, Kauma H (2002): Hypophyseal hemorrhage and panhypopituitarism during Puumala Virus Infection: Magnetic Resonance Imaging and detection of viral antigen in the hypophysis. *Clin Infect Dis;* 35(1):96-101.

Hautala T, Hautala N, Mähönen SM, Sironen T, Pääkkö E, Karttunen A, Salmela PI, Vainio O, Rytky S, Plyusnin A, Vaheri A, Vapalahti O, Kauma H (2011): Young male patients are at elevated risk of developing serious central nervous system complications during acute Puumala hantavirus infection. *BMC Infect Dis;* 11:217.

Heiske A, Anheier B, Pilaski J, Klenk HD, Gröne HJ, Feldmann H (1999): Polymerase chain reaction detection of Puumala virus RNA in formaldehyde-fixed biopsy material. *Kidney Int;* 55(5):2062-9.

Heyman P, Ceianu CS, Christova I, Tordo N, Beersma M, João Alves M, Lundkvist A, Hukic M, Papa A, Tenorio A, Zelená H, Essbauer S, Visontai I, Golovljova I, Connell J, Nicoletti L, Van Esbroeck M, Gjeruldsen Dudman S, Aberle SW, Avšić-Županc T, Korukluoglu G, Nowakowska A, Klempa B, Ulrich RG, Bino S, Engler O, Opp M, Vaheri A (2011): A five-year perspective on the situation of haemorrhagic fever with renal syndrome and status of the hantavirus reservoirs in Europe, 2005-2010. *Euro Surveill;* 16(36).

Hjelle B (1998): Virus detection and identification with genetic tests. *In C C;* 132–137.

Hjertqvist M, Klein SL, Ahlm C, Klingstrom J (2010): Mortality rate patterns for hemorrhagic fever with renal syndrome caused by Puumala virus. *Emerg Infect Dis;* 16(10):1584-6.

Hoier S, Aberle SW, Langner C, Schnedl W, Högenauer C, Reisinger EC, Krejs GJ, Krause R (2006): Puumala virus RNA in patient with multiorgan failure. *Emerg Infect Dis;* 12(2):356-7.

Holmes EC, Zhang YZ (2015): The evolution and emergence of hantaviruses. *Curr Opin Virol;* 10:27-33.

Hukic M, Nikolic J, Valjevac A, Seremet M, Tesic G, Markotic A (2010): A serosurvey reveals Bosnia and Herzegovina as a Europe's hotspot in hantavirus seroprevalence. *Epidemiol Infect*;138(8):1185-93.

Hung T, Zhou JY, Tang YM, Zhao TX, Baek LJ, Lee HW (1992): Identification of Hantaan virus-related structures in kidneys of cadavers with haemorrhagic fever with renal syndrome. *Arch Virol;* 122(1-2):187-99.

International Committee on Taxonomy of Viruses. Index of viruses-*Bunyaviridae*, 2006. Available online: http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs_index.htm

Jakab F, Horváth G, Ferenczi E, Sebok J, Varecza Z, Szucs G (2007): Detection of Dobrava hantaviruses in Apodemus agrarius mice in the Transdanubian region of Hungary. *Virus Res;* 128(1-2):149-52.

Jakab F, Sebok J, Ferenczi E, Horváth G, Szucs G (2007): First detection of Dobrava hantavirus from a patient with severe haemorrhagic fever with renal syndrome by SYBR Green-based real time RT-PCR. *Scand J Infect Dis;* 39(10):902-6.

Jakab F, Horváth G, Ferenczi E, Sebők J, Szűcs G (2008): First detection of Tula hantaviruses in Microtus arvalis voles in Hungary. *Arch Virol;* 153(11):2093-6.

Jakab F, Sebok J, Szántó Z, Hang D, Imre M, Németh V, Madai M, Oldal M, Kovács T, Wittmann I (2011): Dobrava-Belgrade hantavirus infection mimics acute appendicitis. *J Clin Virol;* 50(2):164-6.

Jonsson CB, Gallegos J, Ferro P, Severson W, Xu X, Schmaljohn CS (2001): Purification and characterization of the Sin Nombre virus nucleocapsid protein expressed in Escherichia coli. *Protein Expr Purif;* 23(1):134-41.

Johnson KM (2004): The discovery of hantaan virus: comparative biology and serendipity in a world at war. *J Infect Dis;* 190(9):1708-10.

Jonsson CB, Figueiredo LT, Vapalahti O (2010): A global perspective on hantavirus ecology, epidemiology, and disease. *Clin Microbiol Rev;* (2):412-41.

Kallio-Kokko, H., A. Lundkvist, A. Plyusnin, T. Avsic-Zupanc, A. Vaheri, and O. Vapalahti (2000): Antigenic properties and diagnostic potential of recombinant Dobrava virus nucleocapsid protein. *J Med Virol;* 61:266–274.

Kallio-Kokko H, Leveelahti R, Brummer-Korvenkontio M, Lundkvist A, Vaheri A, Vapalahti O (2001): Human immune response to Puumala virus glycoproteins and nucleocapsid protein expressed in mammalian cells. *J Med Virol;* 65(3):605-13.

Kanerva M, Paakkala A, Mustonen J, Paakkala T, Lahtela J, Pasternack A (1996): Pulmonary involvement in nephropathia epidemica: radiological findings and their clinical correlations. *Clin Nephrol;* 46:369–78.

Khang HJ, Bennett SN, Sumibcay L, Arai S, Hope AG, Mocz G, Song JW, Cook JA, Yanagihara R (2009): Evolutionary insights from a genetically divergent hantavirus harbored by the European common mole (Talpa europaea). *PLoS One;* 4(7):e6149.

Kehm R, Jakob NJ, Welzel TM, Tobiasch E, Viczian O, Jock S, Geider K, Süle S, Darai G (2001): Expression of immunogenic Puumala virus nucleocapsid protein in transgenic tobacco and potato plants. *Virus Genes;* 22(1):73-83.

Khattak S, Darai G, Süle S, Rösen-Wolff A (2002): Characterization of expression of Puumala virus nucleocapsid protein in transgenic plants. *Intervirology;* 45(4-6):334-9.

Kim S, Kang ET, Kim YG, Han JS, Lee JS, Kim YI, Hall WC, Dalrymple JM, Peters CJ (1993): Localization of Hantaan viral envelope glycoproteins by monoclonal antibodies in renal tissues from patients with Korean hemorrhagic fever H. *Am J Clin Pathol;* 100(4):398-403.

Klein SL, Marks MA, Li W, Glass GE, Fang LQ, Ma JQ, Cao WC (2011): Sex differences in the incidence and case fatality rates from hemorrhagic fever with renal syndrome in China, 2004-2008. *Clin Infect Dis;* 52(12):1414-21.

Klempa B, Meisel H, Rath S, Bartel J, Ulrich R, Kruger DH (2003): Occurrence of renal and pulmonary syndrome in a region of northeast Germany where Tula hantavirus circulates. *J Clin Microbiol* 41(10):4894–4897

Klempa B, Radosa L, Kruger DH (2013): The broad spectrum of hantaviruses and their hosts in Central Europe. *Acta Virol;* 57(2):130-7.

Korva M, Saksida A, Kejžar N, Schmaljohn C, Avšič-Županc T (2013b): Viral load and immune response dynamics in patients with haemorrhagic fever with renal syndrome. Clin Microbiol Infect; 19(8):E358-66.

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

Krautkrämer E, Zeier M, Plyusnin A (2013): Hantavirus infection: an emerging infectious disease causing acute renal failure. *Kidney Int;* 83(1):23-7.

Krüger DH, Ulrich RG, Hofmann J (2013): Hantaviruses as zoonotic pathogens in Germany. *Dtsch Arztebl Int;* 110:461-67.

Kryštufek, B (1991): Sesalci Slovenije. Prirodoslovni muzej Slovenije, *Ljubljana*, pp.

150–159.

Lagerqvist N, Hagström Å, Lundahl M, Nilsson E, Juremalm M, Larsson I, Alm E, Bucht G, Ahlm C, Klingström J (2016): Molecular Diagnosis of Hemorrhagic Fever with Renal Syndrome Caused by Puumala Virus. *J Clin Microbiol;* 54(5):1335-9.

Lee HW, Baek LJ, Johnson KM (1982): Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic fever, from wild urban rats. *J Infect Dis;* 146(5):638-44.

Lee HW, Johnson KM (1982): Laboratory-acquired infections with Hantaan virus, the etiologic agent of Korean hemorrhagic fever. *J Infect Dis;* 146(5):645-51.

Lee HW, Schmaljohn C (ed.) Manual of haemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. WHO Collaborating Center for Virus Reference and Research (Hantaviruses), Asian Institute for Life Sciences, Seoul, South Korea.

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

Lin XD, Guo WP, Wang W, Zou Y, Hao ZY, Zhou DJ, Dong X, Qu YG, Li MH, Tian HF, Wen JF, Plyusnin A, Xu J, Zhang YZ (2012): Migration of Norway rats

resulted in the worldwide distribution of Seoul hantavirus today. *J Virol;* 86(2):972-81.

Linderholm M, Billström A, Settergren B, Tärnvik A (1992): Pulmonary involvement in nephropathia epidemica as demonstrated by computed tomography. *Infection;* 20:263–6.

Linderholm M, Sandström T, Rinnström O, Groth S, Blomberg A, Tärnvik A (1997): Impaired pulmonary function in patients with haemorrhagic fever with renal syndrome. *Clin Infect Dis;* 25:1084–9.

Lindkvist M, Lahti K, Lilliehöök B, Holmström A, Ahlm C, Bucht G (2007): Cross-reactive immune responses in mice after genetic vaccination with cDNA encoding hantavirus nucleocapsid proteins. *Vaccine;* 25(9):1690-9.

Lundkvist A, Scholander C, Niklasson B (1993): Anti-idiotypic antibodies against Puumala virus glycoprotein-specific monoclonal antibodies inhibit virus infection in cell cultures. *Arch Virol;* 132(3-4):255-65.

Lundkvist A, Vapalahti O, Plyusnin A, Sjölander KB, Niklasson B, Vaheri A (1996): Characterization of Tula virus antigenic determinants defined by monoclonal antibodies raised against baculovirus-expressed nucleocapsid protein. *Virus Res;* 45(1):29-44.

Maftei ID, Segall L, Panculescu-Gatej R, Ceianu C, Covic A (2012): Hantavirus infection--hemorrhagic fever with renal syndrome: the first case series reported in Romania and review of the literature. *Int Urol Nephrol*; 44(4):1185-91.

Markotić A, Kuzman I, Babić K, Gagro A, Nichol S, Ksiazek TG, Rabatić S, Dekaris D (2002a): Double trouble: hemorrhagic fever with renal syndrome and leptospirosis. *Scand J Infect Dis*; 34(3):221-4.

Markotić A, Nichol ST, Kuzman I, Sanchez AJ, Ksiazek TG, Gagro A, Rabatić S, Zgorelec R, Avsic-Zupanc T, Beus I, Dekaris D (2002b): Characteristics of Puumala and Dobrava infections in Croatia. *J Med Virol*; 66(4):542-51.

Martinez VP, Bellomo CM, Cacace ML, Suarez P, Bogni L, Padula PJ. Hantavirus pulmonary syndrome in Argentina, 1995-2008. *Emerg Infect Dis;* 16(12):1853-60.

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

Meisel H, Wolbert A, Razanskiene A, Marg A, Kazaks A, Sasnauskas K, Pauli G, Ulrich R, Krüger DH (2006): Development of novel immunoglobulin G (IgG), IgA, and IgM enzyme immunoassays based on recombinant Puumala and Dobrava hantavirus nucleocapsid proteins. *Clin Vaccine Immunol;* 13(12):1349-57.

Mertens M, Hofmann J, Petraityte-Burneikiene R, Ziller M, Sasnauskas K, Friedrich R, Niederstrasser O, Krüger DH, Groschup MH, Petri E, Werdermann S, Ulrich RG (2011): Seroprevalence study in forestry workers of a non-endemic region in

eastern Germany reveals infections by Tula and Dobrava-Belgrade hantaviruses. *Med Microbiol Immunol;* 200(4):263-8.

Myhrman G (1951): Nephropathia epidemica a new infectious disease in northern Scandinavia. *Acta Med Scand;* 140(1):52-6.

Nemes Zs, Péterfi Z (2000): [Simultaneous leptospirosis and hantavirus infection in the same patient]. [Article in Hungarian*] Orv Hetil*;141(10):499-502.

Németh V, Madai M, Marászi A, Bérczi B, Horváth G, Oldal M, Kisfali P, Bányai K, Jakab F (2011): Detection of Dobrava-Belgrade hantavirus using recombinant-nucleocapsid-based enzyme-linked immunosorbent assay and SYBR Green-based real-time reverse transcriptase-polymerase chain reaction. *Arch Virol;* 156(9):1655-60.

Németh V, Oldal M, Madai M, Horváth G, Kemenesi G, Dallos B, Bányai K, Jakab F (2013): Molecular characterization of Dobrava and Kurkino genotypes of Dobrava-Belgrade hantavirus detected in Hungary and Northern Croatia. *Virus Genes;* 47(3):546-9.

Padula P, Martinez VP, Bellomo C, Maidana S, San Juan J, Tagliaferri P, Bargardi S, Vazquez C, Colucci N, Estévez J, Almiron M (2007): Pathogenic hantaviruses, northeastern Argentina and eastern Paraguay. *Emerg Infect Dis;* 13(8):1211-4.

Plyusnin A, Vapalahti O, Lankinen H, Lehväslaiho H, Apekina N, Myasnikov Y, Kallio-Kokko H, Henttonen H, Lundkvist A, Brummer-Korvenkontio M, et al. (1994): Tula virus: a newly detected hantavirus carried by European common voles. *J Virol*; 68(12):7833-9.

Plyusnin A, Vapalahti O, Vasilenko V, Henttonen H, Vaheri A (1997): Dobrava hantavirus in Estonia: does the virus exist throughout Europe? *Lancet;* 349(9062):1369-70.

Plyusnin A, Vaheri A, Lundkvist A (2006): Saaremaa hantavirus should not be confused with its dangerous relative, Dobrava virus. *J Clin Microbiol;* 44(4):1608-9; author reply 1609-11.

Plyusnina A, Ferenczi E, Rácz GR, Nemirov K, Lundkvist A, Vaheri A, Vapalathi O, Plyusnin A (2009): Co-circulation of three pathogenic hantaviruses? Puumala, Dobrava and Saaremaa in Hungary. *J Med Virol;* 81(12):2045-52.

Prince HE, Su X, Hogrefe WR (2007): Utilization of hantavirus antibody results generated over a five-year period to develop an improved serologic algorithm for detecting acute Sin Nombre hantavirus infection. *J Clin Lab Anal;* 21(1):7-13.

Radosa L, Schlegel M, Gebauer P, Ansorge H, Heroldová M, Jánová E, Stanko M, Mošanský L, Fričová J, Pejčoch M, Suchomel J, Purchart L, Groschup MH, Krüger DH, Ulrich RG, Klempa B (2013): Detection of shrew-borne hantavirus in Eurasian pygmy shrew (Sorex minutus) in Central Europe. *Infect Genet Evol;* 19:403-10.

Raftery MJ, Kraus AA, Ulrich R, Krüger DH, Schönrich G (2002): Hantavirus infection of dendritic cells. *J Virol;* 76(21):10724-33.

Rasmuson J, Andersson C, Norrman E, Haney M, Evander M, Ahlm C (2011): Time to revise the paradigm of hantavirus syndromes? Hantavirus pulmonary syndrome caused by European hantavirus. *Eur J Clin Microbiol Infect Dis;* 30:685–90.

Rasmuson J, Lindqvist P, Sörensen K, Hedström M, Blomberg A, Ahlm C (2013): Cardiopulmonary involvement in Puumala hantavirus infection. *BMC Infect Dis;* 13:501.

Razanskiene A, Schmidt J, Geldmacher A, Ritzi A, Niedrig M, Lundkvist A, Krüger DH, Meisel H, Sasnauskas K, Ulrich R (2004): High yields of stable and highly pure nucleocapsid proteins of different hantaviruses can be generated in the yeast Saccharomyces cerevisiae. *J Biotechnol;* 111(3):319-33.

Reusken C, Heyman P (2013): Factors driving hantavirus emergence in Europe. *Curr Opin Virol;* 3:92-9.

Scharninghausen JJ, Meyer H, Pfeffer M, Davis DS, Honeycutt RL (1999): Genetic evidence of Dobrava virus in Apodemus agrarius in Hungary. *Emerg Infect Dis;* 5(3): 468-70.

Schmaljohn CS, Dalrymple JM (1983): Analysis of Hantaan virus RNA: evidence for a new genus of bunyaviridae. *Virology;* 131(2):482-91.

Schmaljohn CS, Hasty SE, Harrison SA, Dalrymple JM (1983): Characterization of Hantaan virions, the prototype virus of hemorrhagic fever with renal syndrome. *J Infect Dis;* 148(6):1005-12.

Schmaljohn CS, Sugiyama K, Schmaljohn AL, Bishop DH (1988): Baculovirus expression of the small genome segment of Hantaan virus and potential use of the expressed nucleocapsid protein as a diagnostic antigen. *J Gen Virol;* 69(4):777-86.

Schmidt J, Jandrig B, Klempa B, Yoshimatsu K, Arikawa J, Meisel H, Niedrig M, Pitra C, Krüger DH, Ulrich R (2005): Nucleocapsid protein of cell culture-adapted Seoul virus strain 80-39: analysis of its encoding sequence, expression in yeast and immuno-reactivity. *Virus Genes;* 30(1):37-48.

Schultze D, Fierz W, Matter HC, Bankoul S, Niedrig M, Schmiedl A (2007): Cross-sectional survey on hantavirus seroprevalence in Canton St. Gallen, Switzerland. *Swiss Med Wkly;* 137(1-2):21-6.

Seitsonen E, Hynninen M, Kolho E, Kallio-Kokko H, Pettila V (2006): Corticosteroids combined with continuous veno-venous hemodiafiltration for treatment of hantavirus pulmonary syndrome caused by Puumala virus infection. *Eur J Clin Microbiol Infect Dis;* 25:261–6.

Sibold C, Meisel H, Lundkvist A, Schulz A, Cifire F, Ulrich R, Kozuch O, Labuda M, Krüger DH (1999): Short report: simultaneous occurrence of Dobrava, Puumala, and Tula Hantaviruses in Slovakia. *Am J Trop Med Hyg;* 61(3):409-11.

Sironen, T., Voutilainen, L., Isoviita, V.-M., Niemimaa, J., Vaheri, A., Vapalahti, O., Henttonen, H (2010): Isolation and characterization of novel insectivore-borne hantaviruses from Finland, in: *VIII International Conference on HFRS, HPS & Hantaviruses*, 20–22 May, pp. 186.

Song JW, Baek LJ, Song KJ, Skrok A, Markowski J, Bratosiewicz-Wasik J, Kordek R, Liberski PP, Yanagihara R (2004): Characterization of Tula virus from common voles (microtus arvalis) in Poland: evidence for geographic-specific phylogenetic clustering. *Virus Genes;* 29(2):239-47.

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

Stanojevic M, Nikolic V, Stajkovic N, Stamenkovic G, Bozovic B, Cekanac R, Marusic P, Gligic A (2015): Genetic detection of Dobrava-Belgrade hantavirus in the edible dormouse (Glis glis) in central Serbia. *Epidemiol Infect*; 143(2):400-4.

Suchomel J (2007): Contribution to the knowledge of *Clethrionomys glareolus* populations in forests of managed landscape in Southern Moravia (Czech Republic). *J For Sci;* 53(7):340-44.

Tadin A, Turk N, Korva M, Margaletić J, Beck R, Vucelja M, Habuš J, Svoboda P, Zupanc TA, Henttonen H, Markotić A(2012): Multiple co-infections of rodents with hantaviruses, Leptospira, and Babesia in Croatia. *Vector Borne Zoonotic Dis*; 12(5):388-92.

Tai PW, Chen LC, Huang CH (2005): Hanta haemorrhagic fever with renal syndrome: a case report and review. *J Microbiol Immunol Infect;* 38(3):221-23.

Taylor LH, Latham SM, Woolhouse ME (2001): Risk factors for human disease emergence. *Philos Trans R Soc Lond Series B Biol Sc;* 356(1411): 983–989.

Trencséni T, Keleti B (1971): Clinical aspects and epidemiology of haemorrhagic fever with renal syndrome: analysis of clinical and epidemiological experiences in Hungary. *Akadémiai Kiadó;* 247

Trubenovį K, Miklós P (2007): Population ecology of *Apodemus flavicollis* in a montane beech-spruce forest in the western Tatra mountains. *Acta universitatis Comenianae;* 47(1): 83–90.

Vaheri A, Vapalahti O, Plyusnin A (2008): How to diagnose hantavirus infections and detect them in rodents and insectivores. *Rev Med Virol;* 18(4):277-88.

Vaheri A, Henttonen H, Voutilainen L, Mustonen J, Sironen T, Vapalahti O (2013): Hantavirus infections in Europe and their impact on public health. *Rev Med Virol;* 23:35-49.

Vaheri A, Strandin T, Hepojoki J, Sironen T, Henttonen H, Mäkelä S, Mustonen J (2013): Uncovering the mysteries of hantavirus infections. *Nat Rev Microbiol;* 11(8):539-50.

Vapalahti O, Kallio-Kokko H, Närvänen A, Julkunen I, Lundkvist A, Plyusnin A, Lehväslaiho H, Brummer-Korvenkontio M, Vaheri A, Lankinen H. Human B-cell epitopes of Puumala virus nucleocapsid protein, the major antigen in early serological response. *J Med Virol;* 46(4):293-303.

Vapalahti O, Lundkvist A, Kallio-Kokko H, Paukku K, Julkunen I, Lankinen H, Vaheri A (1996): Antigenic properties and diagnostic potential of puumala virus nucleocapsid protein expressed in insect cells. *J Clin Microbiol;* 34(1):119-25.

ViralZone (http://viralzone.expasy.org/all_by_species/213.html)

Virus taxonomy: 2013 release. International comittee on taxonomy of viruses, 2014. (http://www.ictvonline.org/virusTaxonomy.asp)

Vukićević-Radić O, Matic R, Kataranovski D, Stamenković S (2006): Spatial organization and home range of *Apodemus flavicollis* and *A. agrarius* on mt. Avala, Serbia. Acta Zool Acad Sci Hung 52:81–96.

Walter CT, Barr JN (2011): Recent advances in the molecular and cellular biology of bunyaviruses. *J Gen Virol;* 92:2467–2484.

Weidmann M, Schmidt P, Vackova M, Krivanec K, Munclinger P, Hufert FT (2005): Identification of genetic evidence for dobrava virus spillover in rodents by nested reverse transcription (RT)-PCR and TaqMan RT-PCR. *J Clin Microbiol;* 43(2):808-12.

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

Zelená H, Mrázek J, Kuhn T (2013): Tula hantavirus infection in immunocompromised host, Czech Republic. *Emerg Infect Dis;* 19(11):1873-5.

10. List of publications

10.1 Publications related to thesis topic

1. **Oldal M**, Németh V, Madai M, Pintér R, Kemenesi G, Dallos B, Kutas A, Sebők J, Horváth G, Bányai K, Jakab F (2014): Serosurvey of pathogenic hantaviruses among forestry workers in Hungary. *Int J Occup Med Environ Health;* 27(5):766-73.
2. **Oldal M**, Németh V, Madai M, Kemenesi G, Dallos B, Péterfi Z, Sebők J, Wittmann I, Bányai K, Jakab F (2014): Identification of hantavirus infection by Western blot assay and TaqMan PCR in patients hospitalized with acute kidney injury. *Diagn Microbiol Infect Dis;* 79(2):166-70.
3. Németh V, **Oldal M**, Sebők J, Wittmann I, Jakab F (2014): Hantavírus fertőzések hazai jelentősége a legújabb virológiai, epidemiológiai és klinikai vizsgálatok eredményeinek tükrében. *Hypertonia és nephrologia;* 18:(3-4)76-81.

10.2 Oral and poster presentations related to thesis topic

1. **Oldal M**, Németh V, Madai M, Kemenesi G, Horváth G, Bányai K, Jakab F (2014): „*Hantavirus seroprevalence among forestry workers in Hungary*." International Meeting on emerging Diseases and Surveillance; Vienna, Austria October 31 – November 3, 2014., poszter.
2. Jakab F, **Oldal M**, Németh V, Madai M, Kemenesi G, Dallos B, Péterfi Z, Sebők J, Bányai K, Wittmann I (2013): „*Identification of hantavirus infection by Western blot assay and TaqMan PCR in patients hospitalized with acute renal failure".* 4th Central European Forum for Microbiology; Keszthely, October 16-18, 2013., előadás.
3. **Oldal M**, Németh V, Madai M, Dóró R, Kemenesi G, Pintér R, Bányai K, Jakab F (2012): *„Detecting Dobrava-Belgrade and Puumala virus infections in Hungarian forestry workers by ELISA and Western blot analyses."* A Magyar Mikrobiológiai Társaság 2012. évi Nagygyűlése Keszthely 2012. október 24-26., előadás.

10.3 Publications outside thesis topic

1. Antal L, László B, Kotlík P, Mozsár A, Czeglédi I, **Oldal M**, Kemenesi G, Jakab F, Nagy SA (2016): Phylogenetic evidence for a new species of Barbus in the Danube River basin. *Mol Phylogenet Evol*; 96:187-94.
2. Damásdi M, Jakab F, Kovács K, **Oldal M**, Kemenesi G, Szabó E, Vályi-Nagy I, Pytel Á, Farkas L, Szántó Á (2015): Prevalence and Type Diversity of Human Papillomaviruses in Penile Cancers in Hungary. *Pathol Oncol Res*; 22(3):643-6.

3. Kemenesi G, Földes F, Zana B, Kurucz K, Estók P, Boldogh S, Görföl T, Bányai K, **Oldal M**, Jakab F (2016): Genetic Characterization of Providence Virus Isolated from Bat Guano in Hungary. *Genome Announc;*4(3)e00403-16.
4. Kemenesi G, Gellért Á, Dallos B, Görföl T, Boldogh S, Estók P, Marton S, **Oldal M**, Martella V, Bányai K, Jakab F (2016): Sequencing and molecular modeling identifies candidate members of Caliciviridae family in bats. *Infect Genet Evol;* 41:227-32
5. Kurucz K, Kepner A, Krtinic B, Zana B, Földes F, Bányai K, **Oldal M**, Jakab F, Kemenesi G (2016): First molecular identification of Dirofilaria spp. (Onchocercidae) in mosquitoes from Serbia. *Parasitol Res*; 115:(8) pp. 3257-3260.
6. Kurucz K, Kemenesi G, Zana B, Zeghbib S, **Oldal M**, Jakab F (2016): Ecological preferences of the putative West Nile virus vector Uranotaenia unguiculata mosquito with description of an original larval habitat. *North-West J. Zoo* 1:(1) p. 1.
7. Zana B, Kemenesi G, Herczeg R, Dallos B, **Oldal M**, Marton Sz, Krtinic B, Gellért Á, Bányai K, Jakab F (2016): West Nile virus lineage 2 strains possessed NS3249P helicase amino acid mutation during the 2013 outbreak in Serbia "J Vector Borne Di 1:(1) p. 1.
8. Zana B, Kemenesi G, Antal L, Földes F, **Oldal M**, Bányai K, Jakab F (2016): Molecular traces of a putative novel insect flavivirus from Anopheles hyrcanus mosquito species in Hungary ACTA VIROLOGICA 1:(1) p. 1.
9. Kemenesi G, Zhang D, Marton S, Dallos B, Görföl T, Estók P, Boldogh S, Kurucz K, **Oldal M**, Kutas A, Bányai K, Jakab F (2015): Genetic characterization of a novel picornavirus detected in Miniopterus schreibersii bats. *J Gen Virol* 96:(4)815-821.
10. Kemenesi G, Dallos B, Görföl T, Estók P, Boldogh S, Kurucz K, **Oldal M**, Marton S, Bányai K, Jakab F (2015): Genetic diversity and recombination within bufaviruses: Detection of a novel strain in Hungarian bats. *Infect Genet Evol;* 33:288-92.
11. Kemenesi G, Kurucz K, Kepner A, Dallos B, **Oldal M**, Herczeg R, Vajdovics P, Bányai K, Jakab F (2015): Circulation of Dirofilaria repens, Setaria tundra, and Onchocercidae species in Hungary during the period 2011-2013. *Vet Parasitol*; 214(1-2):108-13.
12. Marton S, Mihalov-Kovács E, Dóró R, Csata T, Fehér E, **Oldal M**, Jakab F, Matthijnssens J, Martella V, Bányai K (2015): Canine rotavirus C strain detected in Hungary shows marked genotype diversity. *J Gen Virol*; 96(10):3059-71.
13. Mihalov-Kovács E, Gellért Á, Marton S, Farkas SL, Fehér E, **Oldal M**, Jakab F, Martella V, Bányai K (2015): Candidate new rotavirus species in sheltered dogs, Hungary. *Emerg Infect Dis;* 21:660-663.
14. **Oldal M**, Sironen T, Henttonen H, Vapalahti O, Madai M, Horváth G, Dallos B, Kutas A, Földes F, Kemenesi G, Németh V, Bányai K, Jakab F (2015): Serologic survey of orthopoxvirus infection among rodents in hungary. *Vector Borne Zoonotic Dis;* 15(5):317-22.

15. Dandár E, Farkas SL, Marton S, **Oldal M**, Jakab F, Mató T, Palya V, Bányai K (2014): The complete genome sequence of a European goose reovirus strain. *Arch Virol;* 159:2165-2169.

16. Dandár E, Huhtamo E, Farkas SL, **Oldal M**, Jakab F, Vapalahti O, Bányai K (2014): Complete genome analysis identifies Tvärminne avian virus as a candidate new species within the genus Orthoreovirus. *J Gen Virol,* 95:898-904.

17. Farkas SL, Dandár E, Marton S, Fehér E, **Oldal M**, Jakab F, Mató T, Palya V, Bányai K (2014): Detection of shared genes among Asian and European waterfowl reoviruses in the whole genome constellations. *Infect Genet Evol;* 28C:55-57.

18. Kemenesi G, Dallos B, Görföl T, Boldog S, Estók P, Kurucz K , **Oldal M**, Németh V, Madai M, Bányai K, Jakab F (2014): Novel European lineages of bat astroviruses identified in Hungary. *Acta Virol;* 58:(1)95-98.

19. Kemenesi G, Krtinić B, Milankov V, Kutas A, Dallos B, **Oldal M**, Somogyi N, Németh V, Bányai K, Jakab F (2014): West Nile virus surveillance in mosquitoes, April to October 2013, Vojvodina province, Serbia: implications for the 2014 season. *Euro Surveill;* 19:(16)20779. 5

20. Kemenesi G, Dallos B , **Oldal M**, Kutas A, Földes F, Németh V, Reiter P, Bakonyi T, Bányai K, Jakab F (2014): Putative novel lineage of West Nile virus in Uranotaenia unguiculata mosquito, Hungary. *Virus Disease;* 25:(4)500-503.

21. Kemenesi G, Dallos B, Görföl T, Boldogh S, Estók P, Kurucz K, Kutas A, Földes F, **Oldal M**, Németh V, Martella V, Bányai K, Jakab F (2014): Molecular survey of RNA viruses in Hungarian bats: discovering novel astroviruses, coronaviruses and caliciviruses. *Vector Borne Zoonotic Dis;* 14:(12)846-855.

22. Pintér R, Madai M, Horváth Gy, Németh V, **Oldal M**, Kemenesi G, Dallos B, Bányai K, Jakab F (2014): Molecular detection and phylogenetic analysis of tick-borne encephalitis virus in rodents captured in the Transdanubian region of Hungary. *Vector Borne Zoonotic Dis*; 14:(8)621-624.

23. Papp H, Rigó D, Dán Á, Farkas SL, **Oldal M**, Jakab F, Bányai K (2014): Szokatlan rotavírus antigén kombináció azonosítása fiatal fácánban. *Magyar Állatorvosok Lapja;* 136:729-735.

24. Németh V , **Oldal M**, Madai M, Horváth Gy, Kemenesi G, Dallos B, Bányai K, Jakab F Molecular characterization of Dobrava and Kurkino genotypes of Dobrava-Belgrade hantavirus detected in Hungary and Northern Croatia. *Virus genes;* 47:(3)546-549.

25. Németh V, **Oldal M**, Egyed L, Gyuranecz M, Erdélyi K, Kvell K, Kalvatchev N, Zeller H, Bányai K, Jakab F (2013): Serologic evidence of crimean-congo hemorrhagic Fever virus infection in hungary. *Vector Borne Zoonotic Dis;* 13:(4)270-272.

26. Pintér R, Madai M, Vadkerti E, Németh V, **Oldal M**, Kemenesi G, Dallos B, Gyuranecz M, Kiss G, Bányai K, Jakab F (2013): Identification of tick-borne

encephalitis virus in ticks collected in southeastern Hungary. *Ticks Tick Borne Dis;* 4:(5)427-431.

27. Jakab F, Sebok J, Szanto Z, Hang D, Imre M, Nemeth V, Madai M, **Oldal M**, Kovacs T, Wittmann I (2011): Dobrava-Belgrade hantavirus infection mimics acute appendicitis. *J Clin Virol;* 50:(2)164-166.
28. Németh V, Madai M, Maráczi A, Bérczi B, Horváth Gy, **Oldal M**, Kisfali P, Bányai K, Jakab F (2011): Detection of Dobrava-Belgrade hantavirus using recombinant-nucleocapsid-based enzyme-linked immunosorbent assay and SYBR Green-based real-time reverse transcriptase-polymerase chain reaction. *Arch virol;* 156:(9)1655-1660.
29. Jakab F, Nemeth V, **Oldal M**, Varga L, Nyul Z, Mitchell DK, Matson DO, Szucs G (2010): Epidemiological and clinical characterization of norovirus infections among hospitalized children in Baranya County, Hungary. *J Clin Virol;* 49:(1)75-76.

10.4 Oral and poster presentation abstarcts outside thesis topic

1. **Oldal M**, Sironen T, Henttonen H, Vapalahti O, Madai M, Horváth Gy, Dallos B, Kutas A, Földes F, Kemenesi G, Németh V, Bányai K, Jakab F (2014): „*Tehénhimlő fertőzés szerológiai vizsgálata rágcsálókban Magyarországon*" A Magyar Mikrobiológiai Társaság 2014. évi Nagygyűlése, Keszthely, 2014. október 15-17, előadás.
2. Kemenesi G, **Oldal M**, Dallos B, Kutas A, Földes F, Németh V, Reiter P, Bakonyi T, Bányai K, Jakab F (2014): „*A Nyugat-Nílusi vírus egy feltételezett, új genetikai variánsa Uranotaenia unguiculata szúnyogfajban."* A Magyar Mikrobiológiai Társaság 2014. évi Nagygyűlése, Keszthely, 2014. október 15-17, előadás.
3. Kemenesi G, Dallos B, Görföl T, Boldogh S, Estók P, Kurucz K, Kutas A, Földes F, **Oldal M**, Németh V, Martella V, Bányai K, Jakab F (2014): *„Denevérek által terjesztett RNS vírusok kimutatása: újonnan leírt Astrovírusok, Coronavírusok és Calicivírusok jellemzése."* A Magyar Mikrobiológiai Társaság 2014. évi Nagygyűlése, Keszthely, 2014. október 15-17, előadás.
4. Kemenesi G, Krtinic B, Milankov V, Dallos B, Kutas A**, Oldal M**, Németh V, Bányai K, Jakab F (2014): *„Nyugat-Nílusi Vírus felmérése a vajdaségi szúnyogállomány körében, 2013 áprilistól októberig."* A Magyar Mikrobiológiai Társaság 2014. évi Nagygyűlése, Keszthely, 2014. október 15-17, poszter.
5. Németh V, **Oldal M**, Kemenesi G, Gyuranecz M, Kvell K, Kalvatchev N, Zeller H, Bányai K, Jakab F (2012): *„Krími-Kingói vérzéses láz fertőzés szerológiai igazolása hazánkban."* A Magyar Mikrobiológiai Társaság 2012. évi Nagygyűlése Keszthely 2012. október 24-26., előadás.
6. Madai M, **Oldal M**, Németh V, Horváth Gy, Dóró R, Kemenesi G, Pintér R, Bányai K, Jakab F (2012): *„A cickányok által terjesztett hantavírusok gyakorisága dél-dunántúli területeken csapdázott állatokban."* A Magyar

Mikrobiológiai Társaság 2012. évi Nagygyűlése, Keszthely 2012. október 24-26., előadás.

7. Németh V, Madai M, Maráczi A, Bérczi B , Horváth Gy, **Oldal M**, Kovács T, Bányai K, Jakab F (2010): *„Prevalence of Dobrava-Belgrade and Saaremaa Hantaviruses among Apodemus mice in Hungary and Northern Croatia."* A Magyar Mikrobiológiai Társaság 2010. évi Nagygyűlése, Keszthely 2010. október 13-15., előadás.
8. Németh V, Madai M, Bérczi B, Maráczi A, Horváth Gy, **Oldal M**, Kovács T, Jakab F (2010): „*Seroprevalence and genetic characterization of Dobrava and Saaremaa hantaviruses among rodents (Apodemus agrarius, A. flavicollis) in Hungary and Northern Croatia."* 20th European Congress of Clinical Microbiology and Infectious Diseases, Vienna/Austria, 10 - 13 April 2010.