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Third International Conference on Haemorrhagic Fever with Renal Syndrome (HFRS) and Hantaviruses

J. Mustonen[1], A. Vaheri[2] and J. Clement[3]

[1]Medical School, University of Tampere and Tampere University Hospital, Tampere, Finland; [2]Department of Virology, Haartman Institute, University of Helsinki, Helsinki, Finland; [3]Belgian Zoonosis Workgroup, Queen Astrid Military Hospital, Brussels, Belgium

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Introduction

The *3rd International Conference on HFRS and Hantaviruses* was held in Helsinki, Finland from 31 May to 3 June, 1995. The six main topics of the symposium were: hantavirus ecology and epidemiology, cell interactions and molecular biology, newly discovered hantaviruses, clinical aspects and pathogenesis, laboratory diagnostics and immune response, antiviral and vaccine development.

Ecology and epidemiology

Dr Henttonen (Helsinki, Finland) reviewed population dynamics of rodents. The density fluctuations of the bank vole (*Clethrionomys glareolus*), carrier of the Puumala (PUU) virus, show clear geographic patterns in Europe. PUU virus is the aetiological agent of nephropathia epidemica (NE), the most common form of hantavirus infection in Europe. Most of Scandinavia is characterized by strong population cycles with a frequency of 3–4 years. The peak phases of the vole cycle are characterized by human epidemics of NE. In contrast, in western and central Europe bank voles primarily exhibit a seasonal pattern, increasing annually from spring to autumn and declining in the winter. The average low numbers of NE cases in these areas is probably due to the short duration of annual peak phase in the vole density. It is important to note that the occurrence of most HFRS and NE epidemics can be predicted from the population dynamics of the carrier rodents.

Dr Vapalahti *et al.* (Helsinki, Finland) reported that during the 1990s approximately 1000 cases of serologically verified NE were diagnosed per year in Finland. The seroprevalence of PUU virus IgG antibodies is 5%, which indicates that the number of annual infections is about 4000. The epidemic season of NE in Finland is from November to January, but cases are found throughout the year. Mortality is less than 0.2%.

Dr Hörling *et al.* (Stockholm, Sweden) described distribution and genetic heterogeneity of PUU virus in Sweden. Hantavirus-specific IgG antibodies were detected only in bank voles trapped in northern and central counties, whereas all animals trapped in the southern parts of Sweden were serologically negative. PUU virus detected in lungs from seropositive animals were subjected to direct cycle sequencing. The obtained sequences were all PUU virus type but with a high degree of heterogeneity between different geographical locations. The location of the two distinct PUU virus strains correlates with the postglacial recolonization of bank voles in Sweden, one northern population originating from Finland, and a southern population originating from central Europe.

Dr Vasilenko *et al.* (Tallinn, Estonia) reported that most cases of hantavirus infections in their country are diagnosed in northern Estonia. Most cases are seen during autumn and winter. From 2.1 to 8.1% of blood donor sera are positive for IgG antibodies against three serotypes of hantavirus, mostly the PUU-strain.

Dr Zöller *et al.* (Koblenz, Germany) reported that the seroprevalence of hantavirus antibodies in a total of 13 358 sera from Germany is 1.7%. A prevalence of 3.1% was detected in a low-mountain area in Baden-Württemberg. Occupational risk groups revealed significantly elevated prevalences running from 3.3 to 10%, as did a panel of chronic haemodialysis patients. The PUU serotype was found to be the prevailing virus.

Dr Groen *et al.* (Rotterdam, The Netherlands) reported on the prevalence of hantavirus infections in

Correspondence and offprint requests to: Jukka Mustonen, Medical School, University of Tampere, PO Box 607, FIN-33101 Tampere, Finland.

the Netherlands. More than 10 000 sera were studied. Among individuals with suspected occupational risk, 6% of the animal trappers, 4% of the forestry workers, 2% of the laboratory workers, and 0.4% of the farmers tested were seropositive. The majority of the seropositive individuals lived in rural and forested areas. The main animal reservoir of the infection proved to be the bank vole. Seropositivity was observed in less than 0.1% haemodialysis and renal transplant patients.

Dr Le Guenno (Paris, France) confirmed that the serodiagnosis of HFRS has been available in France since 1982. From this date to the end of 1994, 531 cases have been confirmed. Nearly all cases (520) were detected in the north-eastern part of the country and mainly in the Ardennes, extending to Belgium. The largest epidemic appeared in 1993 with 188 cases. A bank vole is very common in France and is the reservoir of the virus. Almost all cases of HFRS have been caused by the PUU serotype of hantavirus. However, three Seoul (SEO)-induced cases have been confirmed from rural areas, where the responsible vector, the wild rat (*Rattus norvegicus)*, is an agricultural pest.

Dr Rodriguez *et al.* (Barcelona, Spain) reported on seroprevalence of hantavirus infection in Catalonia. Serum samples were taken from 1200 subjects including 280 blood donors and 537 patients undergoing chronic haemodialysis. The definitive prevalence in EIA screening was 0.33%, and the prevalence among haemodialysis patients was 0.37%.

Dr Gligic *et al.* (Belgrade, Yugoslavia) reported that at least four different hantaviruses, Hantaan (HTN), PUU, SEO, and Belgrade virus are responsible for HFRS in Yugoslavia. About 40% of the cases are caused by PUU virus and the remaining 60% are caused by others. Infection with each virus type can result in markedly different severity of illness, with mortality rate from less than 1% to 16%. In addition she reported that currently a large HFRS epidemic is occurring in Bosnia with over 500 cases already.

Dr Borcic *et al.* (Zagreb, Croatia) stated that HFRS has been known in Croatia since 1954, when the first clinical cases were published. The authors have studied with IFA the antigen presence in the main animal reservoirs of hantaviruses. Among the 11 species studied, four were found harbouring the hantaviruses; they were *Apodemus flavicollis* (yellow-necked mouse) 13.3%, *A. agrarius* (field mouse) 8.2%, *Cl. glareolus* (bank vole) 7.5%, and *A. sylvaticus* (wood mouse) 5.2%.

Dr Markotic *et al.* (Zagreb, Croatia) tested sera of 1000 healthy persons for hantavirus infections in Bosnia and Herzegovina in 1989. Of those examined, 5.4% were IgM-EIA positive, showing acute infection. The majority of inapparent cases were in the age group 20–40 years and the seropositive subjects were mostly housewives, workers in wood factories, and farmers. They concluded that inapparent infections are common in the endemic HFRS regions.

Dr Mills *et al.* (Atlanta, USA) reported on the distribution and prevalence of Sin Nombre virus (SNV) in >3000 small mammals of 55 species captured in the south-western USA. SNV or related hantaviruses circulate in most rodent communities. *Peromyscus maniculatus* (deer mouse) is present in all communities, and 3 to 23% are infected with the virus in different parts of the country. Also many other species of rodents were found to be infected with SNV. SNV is a hantavirus associated with a recently described clinical picture, hantavirus pulmonary syndrome (HPS), a form of adult respiratory distress syndrome (ARDS) that appeared lethal in about 50% of the cases.

New hantaviruses

Dr Nichol (Atlanta, USA) reported that numerous novel hantaviruses have been characterized since the identification of HPS in United States in 1993. Over 100 SNV-associated cases have been confirmed. *P. maniculatus* (the deer mouse), *P. leucopus* (the white-footed mouse), and *Sigmodon hispidus* (cotton rat) are the probable primary reservoirs. Other new hantaviruses have been discovered in Brazil, Venezuela, Costa Rica, and Canada.

Dr Hjelle (Alberquerque, NM, USA) announced several new American viruses, of which at least two are genetically novel and associated to distinct rodent hosts: Isla Vista virus carried by a vole, and El Moro Canyon virus carried by a harvest mouse. Moreover, he characterized a serologically and genetically distinctive virus New-York-1 as a novel HPS-agent, carried by white-footed mice.

Dr Plyusnin *et al.* (Helsinki, Finland) reported on a novel hantavirus discovered in European common voles, *Microtus arvalis* and *M. rossiaemeridionalis*. The virus, designated Tula virus (TUL), is a distinct novel member of the genus *Hantavirus* and related to PUU virus. The possible clinical significance of TUL virus is not clear and needs further studies.

Dr Sibold *et al.* (Berlin, Germany) reported on a new hantavirus called Malacky that has been identified in lung tissue specimens of a vole *M. arvalis*. The voles were trapped in West Slovakia (near Malacky town). Malacky represents a new subtype in the newly discovered genetic group TUL. The data show that at least three genetic groups (PUU, HTN, TUL/Malacky) are co-circulating in central Europe.

Dr Pilaski *et al.* (Düsseldorf, Germany) described the first patient in Germany with genetic characterization of a hantavirus causing a severe (but not fatal) human disease, in which, however, all virus isolation attempts remained unsuccessful. The virus termed 'Berkel virus' was closely related to PUU–Sotkamo virus, an isolate from Finland. The data suggest that PUU virus circulating in the western European countries has evolved differently from their north-eastern counterparts.

Clinical picture and pathogenesis

Dr Zaki *et al.* (Atlanta, USA) discussed hantavirus pulmonary syndrome (HPS). The earliest case in their material of 49 patients occurred in 1978. Pulmonary

examination of lung tissue in 44 fatal cases showed an interstitial pneumonitis with mononuclear cell infiltrate and minimal diffuse alveolar damage. Cells composed mainly of a mixture of T lymphocytes and macrophages, but large immunoblasts are considered pathognomonic. Hantaviral antigens were detected by immunohistochemistry (IHC) in most organs and they located predominantly in the capillary microvasculature, with the lung being the primary target organ. The results suggest that the capillary leak syndrome may result from compartmentalization of an antiviral immune response, combined with high levels of hantavirus antigens in the pulmonary microvasculature. In addition to lungs, endothelial immunostaining was observed in all tissues examined. High densities of virus antigens were also observed in lymphoid follicles of the spleen.

Dr Voss *et al.* (Atlanta, USA) have examined several primary and continuous human endothelial cell lines (derived from dermal microvascular, lung microvascular, aorta, coronary artery, pulmonary artery, and umbilical vein) for their susectibility to SNV infection. Among the cells tested, pulmonary cells had more detectable antigen early in infection. The findings support a role for the infection of endothelium by SNV in the development of HPS and in the changes in endothelial cell permeability which lead to the severe pulmonary oedema which characterizes HPS.

Dr Clement *et al.* (Brussels, Belgium) reported that (non-lethal) HPS can also be seen in PUU virus-induced cases of NE. In their material of 60 Belgian NE patients, seven were found with non-cardiogenic acute pulmonary oedema, three of which showed typical bilateral interstitial infiltrates and/or pleural effusions on chest radiography. One patient who presented with a severe ARDS recovered eventually after 20 days of mechanical ventilation.

Dr Mustonen *et al.* (Tampere, Finland) have studied the question of whether the wide spectrum of clinical outcomes in NE is related to the immunological factors in the host by determining the MHC markers in patients with NE. A very high frequency of HLA B8 and DRB1*0301 alleles was found in patients with the most severe course of NE, such as the presence of shock or the need of dialysis therapy.

Dr Ahlm *et al.* (Umeå, Sweden) have studied central nervous system involvement in NE. Cerebrospinal fluid analysis showed elevated levels of IL-6, IFN-γ, neopterin and β_2-microglobulin in most patients as well as increased monocyte count and elevated protein levels in the minority. On MRI examination, eight of 20 patients had cerebral lesions which in general were discrete and unspecific. Three patients had reversible and unspecified pathological patterns of EEG.

Dr Temonen *et al.* (Helsinki, Finland) performed IHC studies on eight kidney biopsies of NE patients. Cell infiltration of monocyte/macrophages and polymorphonuclear leukocytes were found, as well as increased expression of the cytokines TNF-α, TGF-β, and PDGF mainly in the peritubular area at the distal nephron. Concomitantly at the same locations expression of the endothelial adhesion molecules ICAM-1, VCAM, and PECAM was also seen. Interestingly, no IHC evidence of PUU nucleoprotein (NP) was found.

Dr Wiger (Oslo, Norway) reported on three cases of acute NE during pregnancy. A caesarean section was performed in one patient, the other pregnancies progressed normally. The importance of considering hantavirus infection when renal symptoms occur after an acute febrile illness during pregnancy in an endemic area was suggested.

Dr Groeneveld *et al.* (Leiden, The Netherlands) measured serum nitrate concentrations, as an indirect parameter of nitric oxide (NO) production *in vivo* in patients with NE. They found that excessive amounts of NO are produced during the acute phase of the disease and that NO levels correlated with clinical severity. They suggested that NO could play an important role in the pathogenesis of NE.

Dr Linderholm *et al.* (Umeå, Sweden) obtained plasma samples during the acute phase of NE, and assaayed them by EIA for TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, GM-CSF, and IFN-γ. They found TNF-α and IL-6 levels to be elevated in 15/15 and IL-10 in 13/15 patients, with significant correlations to the maximal serum creatinine.

Laboratory diagnosis and immune response

Dr Bautz (Heidelberg, Germany) reviewed the current status on diagnostics. Indirect immunofluorescence using hantavirus-infected cultured cells as antigen, the traditional serological test, is being replaced by IgG and μ-capture IgM enzyme immunoassay (EIA) tests based on the use of recombinant hantavirus antigens. Polymerase chain reaction (PCR), however, seems to have little diagnostic value for diagnosis of human hantavirus infections, since at the time of clinical symptoms it is quite difficult to demonstrate the presence of hantavirus in any human tissues.

Dr Juto (Umeå, Sweden), however, reported on the successful isolation of hantavirus from a severe case of NE.

Several laboratories including those of Dr Bautz, Dr Ulrich (Berlin, Germany), Dr Vapalahti (Helsinki, Finland) and Dr Yoshimatsu (Sapporo, Japan) reported on the diagnostic potential of recombinant hantavirus nucleocapsid (N) proteins. The N protein is the major target in the early IgG response of HFRS and NE patients. Several of the major antigenic epitope clusters, like in many other viruses, are in the variable regions of the protein.

Dr Hjelle (Albuquerque, NM, USA) presented a new recombinant Sin Nombre N- and G_1-protein immunoblot assay as a promising molecular epidemiological tool for studying man–rodent relationships in HPS.

Antiviral and vaccine development

In Korea and China inactivated hantavirus vaccines prepared in suckling mouse brains and rodent cell cultures have been quite widely used against HTN and

SEO virus infections as was reported by Drs H. W. Lee and Cho (Seoul, Korea), Dr Song (Beijing, China) and Dr Rong-Fang (Shanghai, China). In the meantime Dr Schmaljohn (Ft. Detrick, USA) has been developing a vaccinia-vectored HTN virus vaccine for HFRS and now reported on preclinical and phase I clinical trials.

Although there are some reports on the use of ribavirin in hantavirus infections, no new progress was reported and effective antiviral drugs against HFRS and HPS are still missing.

Concluding remarks

The meeting in Helsinki, attended by 150 experts, learned that considerable progress has been made in the fields of HFRS and hantaviruses. However, this was obviously only the beginning, and much work is still needed for effective control and surveillance of hantavirus infections. New hantaviruses are being continuously discovered and apparently relatively little is known about their disease associations. In many areas, such as large areas of central Europe, HFRS is not generally recognized by practising physicians, and diagnostic tests are not widely available. We know very little about the pathogenesis of HFRS and HPS, not much more than that increased capillary permeability appears to be a critical factor. What for example are the roles of cytokines and of cytotoxic T cells in the host response? The next conference, to be held in Atlanta in 1998, may have answers to some of these questions.