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30 HANTAVIRUSES

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SUMMARY

Hantavirus (HTV) is one of the recently discovered (1977) aetiological agents of acute viral haemorrhagic fever, and belongs as such to the group of 'emerging viruses' such as Ebola (1977), Guanarito (1991), and Sabia viruses (1994). HTV is the newest described genus in the Bunyaviridae family, and the only genus in that family that is *not* arthropod-borne, but transmitted by murid rodents.

At least 10 distinct serotypes have now been isolated, and seven more have been genetically characterized (Table 30.1). Of these 17 types of hantaviruses at least eight have been shown to have a clinical significance: Hantaan (HTN) (1977), Seoul (SEO) (1982), Puumala (PUU) (1984), Dobrava (DOB) (1992), Sin Nombre virus (or Four Corners) (SNV) (1993), New York (NYV) (1994), Black Canal Creek (BCC) (1994) and Bayou (BAY) (1995). Each 'serotype' has its own principal rodent vector, its own geographic distribution and its own more or less specific clinical expression. Several other hantaviruses are being evaluated for their public health significance. The majority of HTV serotypes have the kidney as the chief target organ, hence the commonly used official WHO denomination 'haemorrhagic fever with renal syndrome' (HFRS). Thus, acute renal failure in the context of a 'flu-like' illness, particularly when accompained by thrombocytopenia and/or eye symptoms, should evoke the diagnosis. However, Sin Nombre virus (SNV), isolated after a 1993 epidemic in the United States, seems to affect primarily the lungs, where it causes an often lethal form of adult respiratory distress syndrome, now called hantavirus pulmonary syndrome (HPS). The three other 'SNV-like' viruses NYV, BCC, and BAY, seem also to target primarily the human lung, whereas the newly discovered (1994) European strain Tula (TUL), has no known pathogenicity so far.

Infected rodents remain apparently healthy, but have a probably life-long capacity to shed infectious HTV in their excreta. Recent data point to the growing importance of the wild rat as a vector for hitherto unrecognized forms of hantavirus disease (HTVD) on a worldwide scale. Man is the only known disease endpoint of infection, and transmission occurs mainly via inhalation of aerosolized viral particles in open field or in laboratory conditions. Diagnosis can be secured through serological demonstration of specific HTV antibodies. Newer techniques for viral nucleic acid or antigen detection in human and rodent tissues by PCR genotyping or immunohistochemistry have recently been introduced.

Except for intravenous ribavirin in some forms, there is no virus-specific therapy for these diseases, but acute dialysis and/or mechanical ventilation may be indicated in severe cases. Vaccines based on inactivated virus or vectored recombinant viral genes are in development or in field trial phases.

INTRODUCTION

Until 1993, hantavirus disease (HTVD) could be considered as the prototype of a 'new' viral affliction: unknown by many physicians, ill-known even by many virologists, HTVD was considered a rare, somewhat arcane zoonosis with an exotic flavour, being primarily of interest to nephrologists and rodent specialists. However, explosive fresh evidence in 1993 of epidemics in Europe and particularly in the United States has focused worldwide attention on these viruses. It became clear that we were dealing with a rapidly expanding viral zoonosis of global concern.

During these recent epidemics, it also became apparent that physicians were confronted not with a 'new' virus, altered by point mutations and/or genetic reassortment (as can be seen in influenza or in rotaviruses), but rather with an agent having an already archaic presence in its rodent vectors, which had surfaced due to ecological disturbances in the local rodent population (Kilbourne 1990; Childs *et al.* 1994; Hjelle *et al.* 1994*a*, 1995*d*; Spiropoulou *et al.* 1994). Strictly speaking, it is better to refer to the recent hantaviral outbreaks not as a 'new' disease, but rather as a 'newly emerging disease', or as a 'newly recognized disease' (Clement *et al.* 1997).

Table 30.1 Major hantavirus serotypes[a] in chronological order of isolation

Hantavirus serotype (year + reference)	Main rodent vector (geographical spread)	Human illness (type of spread)
1. Thottapalayam (TPM)[b] (1971; Carey *et al.* 1971)	*Suncus murinus* (shrew) (?) (India)	Not recorded
2. Hantaan 76–118 (HTN) (1977; H. W. Lee *et al.* 1978), (1994; Avsic-Zupanc *et al.* 1994)	*Apodemus agrarius* (field mouse) (Asia, eastern Russia and southern Europe) (Slovenia)	Severe: KHF, EHF, HFRS (rural)
3. Prospect Hill (PH) (1982; P. W. Lee *et al.* 1982)	*Microtus pennsylvanicus* (meadow vole) (USA) and *Microtus* sp. (Russia)	Not recorded
4. Seoul (SEO) (1982; H. W. Lee *et al.* 1982; G. Song 1982*b*)	*Rattus norvegicus* (brown rat) (worldwide)	Intermediate HFRS (urban and rural)
5. Puumala (PUU) and *et al.* (POZ-M1) (1984; Niklasson and LeDuc 1984)	*Clethrionomys glareolus* (red bank vole) (Eurasian continent)	Mild: NE, HTVD (rural)
(1990; Diglisic *et al.* 1994)	*Mus musculus* (house mouse) (Serbia)	Severe HFRS
(Chumakov *et al.* 1981)	*C. rutilus* (western Russia)[c]	NE
(Kariwa *et al.* 1995)	*C. rufocanus* (northern Japan)[c]	Not recorded
6. Thailand (THAI) (1985; Elsell *et al.* 1985)	*Bandicota indica* (bandicoot) (Thailand)	Not recorded
7. Dobrava (DOB) (1992; Avsic-Zupanc *et al.* 1992) (1992; Gligic *et al.* 1992)	*Apodemus flavicollis* (yellow necked field mouse) (ex-Yugoslavia)	Very severe HFRS (rural?)
8. Sin Nombre virus (SNV) (1993; Elliott *et al.* 1994) (1993; Schmaljohn *et al.* 1995*a*)	*Peromyscus maniculatus* (deer mouse) (south-western USA)	Often lethal HPS
9. Tula (TUL)[c] (Plyusnin *et al.* 1994)	*Microtus arvalis* (common vole) *Microtus rossiameridionalis* (Russia, Czechia, and Slovakia)	Not recorded
10. New York (NYV) (1994; J. W. Song *et al.* 1994)	*Peromyscus leucopus* (white-footed mouse) (eastern USA, Canada)	HPS
11. El Moro Canyon (ELMC)[c] (Hjelle *et al.* 1994*d*)	*Reithrodontomys megalotis* (western harvest mouse) (USA, Mexico, Canada)	Not recorded
12. Black Creek Canal (BCC) (1994; Rollin *et al.* 1995)	*Sigmodon hispidus* (cotton rat) (eastern and southern USA to Venezuela, Peru)	HPS
13. Rio Segundo (RIOS)[c] (Hjelle *et al.* 1995*a*)	*Reithrodontomys mexicanus* (?) (Mexican harvest mouse) (South America, Costa Rica, Mexico)	Not recorded
14. Bayou (BAY)[c] (Morzunov *et al.* 1995) (Torrez-Martinez and Hjelle 1995)	*Oryzomys palustris* (rice rat) (Louisiana)	HPS
15. Isla Vista (ILV)[c] (W. Song *et al.* 1995*a*)	*Microtus californicus* (California meadow vole) (California, Oregon, Baja Cal., Mexico)	Not recorded
16. Bloodland Lake (BLLL)[c] (W. Song *et al.* 1995*b*)	*Microtus ochrogaster* (prairie vole) (Mid-West USA, southern Canada)	Not recorded
17. Rio Mamore (RMV)[c] (Hjelle *et al.* 1996)	*Oligoryzomys microtis* (small-eared rice rat) (Bolivia)	Not recorded

EHF, Epidemic haemorrhagic fever; HFRS, haemorrhagic fever with renal syndrome (current WHO denomination); HPS, hantavirus pulmonary syndrome; HTVD, hantavirus disease (proposed new common denomination); KHF, Korean haemorrhagic fever; NE, nephropathia epidemica.

[a] A strain of a 'serotype' is neutralized to more than 50 per cent in plaque reduction neutralization tests (PRNT) by homologous antisera. A 'serotype' is thus defined as having no cross-reactions in PRNT with other serotypes or having a homologous to heterologous titre ratio of >1/16 in both directions.

[b] TPM was first considered a novel arbovirus and only recently genetically confirmed as a distinct hantavirus (Xiao *et al.* 1994). For the serotypes 1 through 8 listed here, a perfectly similar division in eight distinct lineages was found by polymerase chain reaction (PCR) genotyping (see text and Fig. 30.1).

[c] To date, these viruses have not been isolated, but most often only characterized by molecular genetic analysis.

(?) = uncertainty about this species being the primary host (see text).

HISTORY

HTV epidemics have been described under various (mostly geographic) denominations, resulting in up to 60 synonyms (Gajdusek 1962). HTVD has an impressive military past, going back probably as far as the American Civil War and the First World War (Brown 1916; Bradford 1916), throughout the Second World War (Stuhlfauth 1943) and other armed conflicts (Fischer-Hoch and McCormick, 1985; Clement 1987*a*).

Paradoxically, Western medicine first 'discovered' HTVD under its severe Far-Eastern form, later called 'Korean haemorrhagic fever' (KHF), during yet another armed conflict in the early 1950s, the Korean War. US Army physicians were suddenly confronted with an up to then unknown acute febrile illness with multiorgan dysfunction (mainly shock, acute renal failure, and haemorrhage), with a mortality rate between 10 and 15 per cent, and affecting over 3000 United Nations troops (Earle 1954). Despite an enormous investigative effort by a special Hemorrhagic Fever Commission of the US Army, it was not until 1976 that H. W. Lee, P. W. Lee, and K. Johnson discovered a virus-specific antigen in the lungs of a Korean striped field mouse (*Apodemus agrarius coreae*), which subsequently led to the isolation and characterization of the responsible agent in 1977, the same year as Ebola virus was first identified (H. W. Lee *et al.* 1978). This first prototype agent was called Hantaan (HTN), after the river which runs near to the famous 38th parallel between North and South Korea, where most of the battles were fought, but also where most of the KHF cases were recorded, and where the HTN-infected rodents were trapped. Thanks to the diligent efforts of the Hemorrhagic Fever Commission, however, an unique collection of more than 600 sera from 245 soldiers admitted with KHF between December 1951 and August 1954, was preserved for posterity, lyophilized in glass ampoules, and labelled with the patient's name, date of onset of disease, and of sampling. This epidemiological treasury, neatly packed in cardboard boxes in three metal trunks, was reopened in 1990, rehydrated and tested for IgM- and IgG-specific antibodies to Hantaan (HTN) and other hantaviral serotypes. Of these patients, 94 per cent (230/245) possessed HTN antibodies, and most sera contained high titred IgM on admission (LeDuc *et al.* 1990). Thus the clinical diagnosis of KHF in hundreds of patients was confirmed by modern and reliable serological techniques more than 40 years after samples were taken, a unique accomplishment in serology.

A further important contribution in recognition in the early 1960s of HTVD as a worldwide problem was the extensive bibliographic and comparative research of D. C. Gajdusek (1962), relating disease in one part of the world with that in another. With the isolation of new serotypes from the early 1980s onward (Table 30.1), allowing serological comparison between proven human cases with Scandinavian so-called 'nephropathia epidemica' (NE) or with Asian KHF, it finally became evident that both infections were caused by an antigenically similar, but not identical HTV (H. W. Lee *et al.* 1979; Svedmyr *et al.* 1980). In a disease bedevilled by a multiple terminology, the revised bibliography of Gajdusek *et al.* (1987) was all the more valuable. A general denomination such as 'hantavirus disease' (HTVD) (Desmyter *et al.* 1984) could now end the confusion. Indeed the hitherto official WHO denomination of 'haemorrhagic fever with renal syndrome' (HFRS) is more and more confusing to the clinician, since 'haemorrhagic' complications are rare or absent in the European and the current American forms of HTVD, whereas a 'renal syndrome' is rather the exception than the rule in the American forms.

THE AGENT

MORPHOLOGY, MOLECULAR BIOLOGY, AND TAXONOMY

Hantaviruses exhibit somewhat greater polymorphism than the other members of the Bunyaviridae family. Round and oval forms are most frequently visualized in electron microscopy, having a mean diameter of 122 nm, but with large variations in size from 78 to 210 nm (Tao *et al.* 1985, 1987). The virus is lipid-enveloped, with regular hollow surface projections. Like other lipid-enveloped viruses, HTVs are susceptible to most disinfectants, e.g. phenolics, dilute hypochlorite solutions, detergents, 70 per cent alcohol, and most general-purpose household disinfectants.

HTVs possess a tripartite, single-stranded, negative-sense RNA genome. These three segments are designated as small (S), medium (M), and large (L), and encode respectively the nucleocapsid (N) protein of 50–53 kDa, the two envelope proteins (G1 and G2) of 65–74 kDa and 55–60 kDa respectively, and a virion-associated polymerase of ± 200 kDa (Schmaljohn *et al.* 1985, 1986, 1987). G1 and G2 are highly glycosylated and have an important role in inducing neutralizing antibodies, used in the plaque reduction neutralization test (PRNT), which is the most sensitive serological technique for differentiating HTVs. Based on PRNT and other serological relationships, at least five distinct groups had been proposed until recently, each carried by a specific main rodent vector, and each having its own geographical distribution: Hantaan (HTN), Seoul (SEO), Thailand (THAI), Puumala

(PUU), and Prospect Hill (PH) (Chu *et al.* 1994) (Table 30.1).

Recent advances in genetic molecular biology have confirmed this classification: by comparing partial nucleotide sequences of a 333 base pair (bp) region which was amplified from the medium (M) genome segment by reverse transcriptase polymerase chain reaction, (RT–PCR), a consensus phylogenetic tree for 30 hantaviruses could be constructed (Xiao *et al.* 1994), which showed the same five lineages (HTN, SEO, THAI, PUU, and PH) as the serotypes already known before (Fig. 30.1). Moreover, a sixth distinct lineage was found (Xiao *et al.* 1994), close to, but not identical with HTN, represented by Dobrava (DOB) virus, isolated from an *Apodemus flavicollis* in Slovenia (Avsic-Zupanc *et al.* 1992) and isolated also from patients in Serbia under the name Belgrade (Gligic *et al.* 1992).

Molecular genetic analysis has proved to be a valuable tool, complementing and extending serology for classification of new hantaviruses. It is also striking to see the utility of this analysis in demonstrating the similar topology of the virus phylogenetic dendrogram to that of the rodent hosts. This provides strong evidence of the close and ancient relationship of the virus to the rodent, and confirms again the important role of the rodent–man link in HTV disease.

By amplifying in RT–PCR a 241 bp region of the small (S) segment of Thottapalayam (TPH) virus, a distantly related HTV isolated in 1971 from a shrew captured in India (Carey *et al.* 1971), a seventh lineage was found, different from 13 other HTV isolates examined by the same technique (Xiao *et al.* 1994). It is interesting to note that TPH is not only genetically distinct from all other known HTVs, but that also its geographic origin (India) and its host (an insectivore rather than a murid rodent) are totally different from other known hantaviruses (Table 30.1, Fig. 30.1).

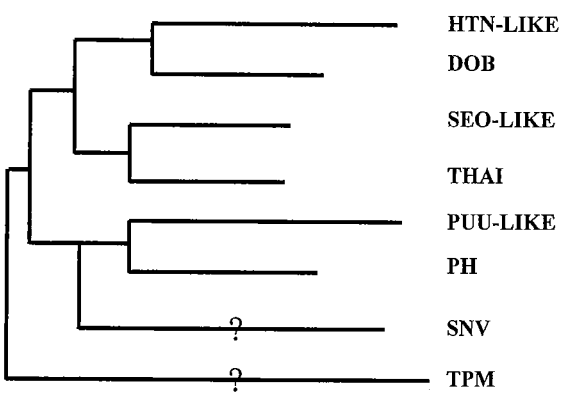

Fig. 30.1 Phylogram summarizing in a very schematic way the data of 32 different HTV isolates. This is a consensus tree based mainly on 100 bootstrap replications of the 333 bp M segment nucleotide sequences (from Xiao *et al.* 1994, with permission from the author). For the sake of completion, TPM and the recently isolated SNV have been added. Since different techniques have been used for their genotyping (see text), comparative branch lengths of TPM and SNV cannot be used, hence the question marks. Vertical distances are for visual clarity only. The 32 isolates have been grouped into eight different serotypes (HTN, DOB, SEO, THAI, PUU, PH, SNV, TPM). For the explanation of abbreviations, see Table 30.1).

The most spectacular demonstration of the possibilities of genetic molecular biology, however, was given during the outbreak of the mysterious epidemic of acute respiratory failure in south-western USA, later to be called 'hantavirus pulmonary syndrome' (HPS). A mere 3 months after the discovery of the first fatal HPS cases, Nichol *et al.* (1993) submitted to *Science* a report, describing part of the G2-encoding M genome of viral RNA extracted from human *and* rodent tissues, and amplified by RT–PCR. Thus, almost from the start a genetic link was made to the responsible rodent vector, the deer mouse *Peromyscus maniculatus*. Nucleotide sequences established for 278 bp differed from that of any of the known HTVs by at least 30 per cent and indicated that the 'HPS agent' was a novel HTV, representing a new eighth lineage, first called four Corners virus (FCV) or Muerto Canyon virus (MCV), and later coined Sin Nombre virus (SNV) (Table 30.1, Fig. 30.1). Subsequent complete sequencing of all three RNA segments has shown that each segment of the new virus is independent of previously known hantaviruses and occupies the same evolutionary topology in relation to the other known viruses, demonstrating that SNV is not a re-assortant virus (Hjelle *et al.* 1994*a*; Spiropoulou *et al.* 1994) Further PCR-typing confirmed this genetic diversity of SNV, with geographic clines of distinct genotypes all across the United States, suggesting that HPS and associated viruses had existed in North America for many years (Childs *et al.* 1994; Hjelle *et al.* 1994*a,b,c* 1995*d*; Spiropoulou *et al.* 1994).

The surprising finding of a new North American HTV was strengthened very quickly by the description of almost identical M-genome nucleotide sequences in samples from *Peromyscus maniculatus*, collected in 1983 (Nerurkar *et al.* 1993) and the retrospective diagnosis from autopsy tissues of a HPS case who died from respiratory failure in 1983 (Zaki *et al.* 1994). All this was achieved before even the responsible agent was actually isolated in November 1993 (Elliott *et al.* 1994; Schmaljohn *et al.* 1995*a*): a genetic molecular *tour de force*, that was aptly described as 'virology without a virus' (Marshall and Stone 1993). This outbreak of highly lethal adult respiratory distress syndrome (ARDS) in relatively young, previously healthy persons thus launched in the United States one of the most intensive, medical sleuth programmes in recent history.

As of August 1997, a total of 172 cases of HPS have been reported in United States, with an overall mortality of ±45.3 per cent (ProMED-mail on 4 Sept'97 12.00 pm).

Four other new human pathogenic HTVs have been identified in the Americas, distinct from SNV. Three were inferred from genetic sequences detected by RT–PCR in lung tissue from deceased HPS patients. One of these cases succumbed to HPS after a probable exposure on Shelter Island, New York, i.e. outside the normal habitation range of the suspected rodent vector *P. maniculatus*. M genomic RT–PCR analysis of necropsy lung tissue differed by only 1.1 per cent from the amplicon obtained from white-footed mice (*P. leucopus*), trapped on Shelter Island, but both amplicons differed by 23 per cent from 'classical' SNV (Hjelle *et al.* 1995*b,c*). Indeed, a distinctive non-SNV HTV could later be isolated from white-footed mice, and is now called New York virus (NYV) (J. W. Song *et al.* 1994).

Another patient died in shock, ARDS, and acute renal failure (ARF) in Louisiana, again a region where *P. maniculatus* is not present (Khan *et al.* 1995). Although this virus has not been isolated so far, sequence analysis and expression of the N protein reveal it to be a new hantavirus, now referred to as Bayou (BAY), and related to, but distinct from SNV (Morzunov *et al.* 1995). Genotypic comparison with RT–PCR products obtained from rice rats (*Oryzomys palustris*), formerly trapped in southern Louisiana, pointed to these rodents as the most likely natural reservoir (Torrez-Martinez and Hjelle 1995).

A third case died in Brazil with classic HPS, and limited sequence analysis of RNA extracted from the lung and amplified by RT–PCR showed the presence of a distinct virus (Khabbaz, 1994).

A fourth patient recovered from hypotension, ARDS, and ARF in Florida, again a region outside the normal biosphere of *P. maniculatus*. This last patient remained serologically inconclusive when tested against SNV antigen, but 12 (13 per cent) of 90 cotton rats (*Sigmodon hispidus*), trapped in his living area, were HTV seropositive. PCR genotyping from their lungs resulted in nucleotide sequences related to, but distinct from, SNV and BAY virus (MMWR 1994*a*). Once the virus, called Black Creek Canal virus (BCC), was isolated and used as antigen, IgM serology of this patient became positive (Rollin *et al.* 1995). Interestingly, a slight cross-reactivity was also noted in recombinant Western blot format between the G1 antigen of BCC and SNV (Hjelle *et al.* 1994*b*). Extensive sequence analysis has confirmed it as a new species of HTV with no evidence of re-assortment with SNV or BAY (Ravkov *et al.* 1995). Thus, rapidly accumulating evidence points to different HTV agents and different New World murid rodent vectors inducing HPS (Fig. 30.2).

Finally, several not previously described HTV were discovered by PCR cloning in the European common voles *Microtus arvalis* and *M. rossiameridionalis*, first trapped in the Tula region, south of Moscow (Plyusnin

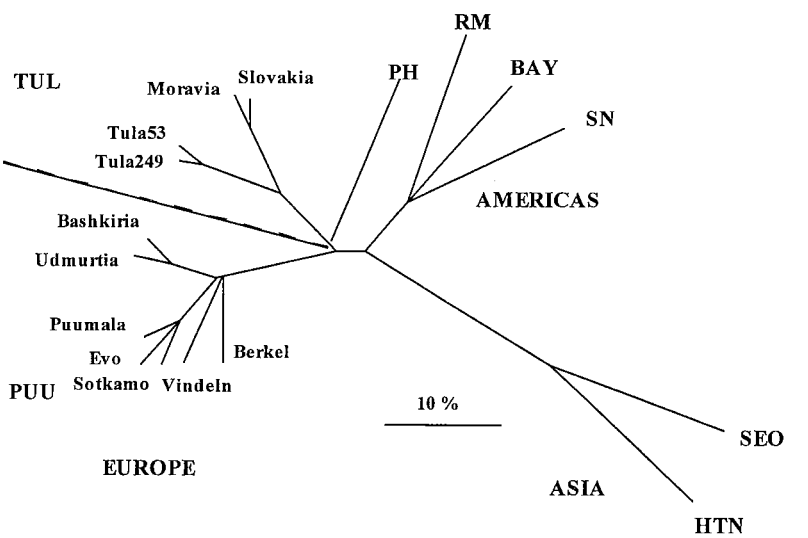

Fig. 30.2 Phylogenetic tree of hantaviruses based on S segment nucleotides, with their geographic distribution. PUU, Puumala virus; TUL, Tula; PH, Prospect Hill; RM, hantavirus carried by *Reithrodontomys megalotis* (western harvest mouse) now called El Moro Canyon virus (ELMC); BAY, Bayou; SN, Sin Nombre; SEO, Seoul; HTN, Hantaan. Tula53 and Tula249 are examples of two strains derived from the same location. Other names refer to other European PUU and TUL hantavirus strains. The bar length indicates the approximate length of a 10 per cent difference in the S segment nucleotide sequences.

et al. 1994), later also in Moravia, Czech Republic (Plyusnin *et al.* 1995) and in Slovakia (Sibold *et al.* 1995). All these central European HTV appeared related to, but distinct from PUU, and were designated as Tula virus (TUL) (Table 30.1, Fig. 30.2). So far, TUL has no known human pathogenecity, but a high degree of serological cross-reaction has been observed between TUL and PUU nucleocapsid antigen, both for IgM and for IgG, so that human TUL infections could have been incorrectly diagnosed as PUU in the past (Niklasson *et al.* unpublished observation). Moreover, it is of great interest that TUL maps on the phylogenetic tree close to (non-pathogenic) Prospect Hill (PH) and the other (very pathogenic) American HTVs (Fig. 30.2) (Clement *et al.* 1997).

GROWTH

HTVs are notoriously difficult to isolate and to grow in culture; several blind passages are frequently necessary, and the results are difficult to judge since a cytopathogenic effect (CPE) is most often lacking. This is even more true for PUU isolations where supernatants are often of low titres. Moreover, the viraemic phase seems to be very short, particularly in PUU infections, explaining perhaps the frequently negative isolation attempts in PUU infections, even when PCR products clearly indicate the presence of a PUU-like RNA (Pilaski *et al.* 1995). Thus, diagnosis has to rely on serology or on newer techniques (see p. 343–4) (Clement *et al.* 1995). The prototype Korean isolate Hantaan (HTN) was first adapted to tissue culture by French *et al.* (1981) using the A-549 cell line, derived from a human lung carcinoma. Growth in Vero E6 culture, a continuous kidney cell line from the African green monkey, resulted in development of a satisfactory plaque-reduction neutralization test (PRNT) and sufficiently high titred virus growth for further characterization (McCormick *et al.* 1982).

TRANSMISSION

HTV is carried to man by excretions and aerosols, consisting of infected respiratory and urinary droplets from apparently healthy rodent carriers, who excrete the virus in saliva, in urine, in faeces, and from their lungs. The survival time of HTVs in the environment in liquids or aerosols or in a dried state is not known.

Whereas most of proven HTVD patients actually can recall sighting of rodents, both in Europe (Clement *et al.* 1994*d*, 1996) and in the United States (Zeitz *et al.* 1995), a physical contact with rodents is almost never mentioned (Dournon *et al.* 1984), except in laboratory conditions (Desmyter *et al.* 1983). Epidemiological studies in farm workers in China (Xu *et al.* 1985) and in shepherds and woodcutters in Greece (Antoniadis *et al.* 1987*b*) revealed a high infection rate among individuals sleeping on the ground, a finding that was confirmed in a case-control study among US military in the region of Ulm (Southern Germany), after an 1990 outbreak nephropathia epidemica during a winter field exercise (Clement *et al.* 1996). In the United States most cases are rural in origin and often have a history of agricultural activities or cleaning outbuildings; there is a correlation with increased rodent populations locally (Zeitz *et al.* 1995). Infections may well be acquired indoors in many cases because of the propensity of the major reservoir, *Peromyscus maniculatus*, to enter houses (Childs *et al.* 1994). On a worldwide scale, the same may be true for the wild rat (*Rattus norvegicus*), an often underestimated source of indoor HTV infections (G. Song *et al.* 1982*b*; Clement *et al.* 1994*d*; McKenna *et al.* 1994).

Intercage airborne transmission of HTN virus between laboratory cages of *Apodemus* sp., housed 1–4 m apart, was demonstrated by H. W. Lee *et al.* (1981), whereas Gavrilovskaya *et al.* (1990) showed airborne transmission of a PUU-like virus between cages of bank voles 1.5 m apart. In a laboratory setting, 12–16 week old Wistar rats appeared susceptible to aerosol exposure of extremely small amounts of HTN, SEO, and PUU viruses (Nuzum *et al.* 1988). The high aerosol infectivity of hantaviruses requires special precautions in the virology laboratory, particularly if viral concentrates or infected animals are involved. The substantial mortality of SNV infections has led to the recommendation that the latter be performed only at biosafety level 4 (MMWR 1994*b*). In addition, laboratory manipulations involving the introduction of rat-derived immunocytomas or hybridomas originating from HTV-infected rat colonies into HTV-free colonies has also been shown to represent an efficient means of HTV transmission among laboratory animals (McKenna *et al.* 1992).

Contamination of food (particularly by wild rats) and direct contact with rodent excreta through skin abrasions may be another occasional route of infection (Kopela and Lähdevirta 1978, Xu *et al.* 1985). Person to person transmission, or infection of personnel nursing HTVD cases, including HPS, has never been documented so far. The fact that outbreaks of HTVD can be extremely localized in circumscribed foci or even microfoci (Clement *et al.* 1996) has prompted the concept of a 'place disease' (Smadel 1953), although it must be recognized that 90 per cent of cases are solitary. There is an important need to understand the dynamics of rodents and viral infection in rodents that leads to these findings.

Interestingly, HTV isolates were also obtained from mites and/or fleas parasitizing *Apodemus* and *Clethrio-*

nomys species in China (Chen and Qiu, 1993). However, the role of these arthropods in the transmission of HTVD is not clear, and to our knowledge this finding has not been reported so far from the West.

PATHOPHYSIOLOGY

Remarkably little is known of the ways by which HTVs can cause multisystem illness and—at least in some severe cases—a multiple organ dysfunction syndrome (MODS) or even a multiorgan failure. It is clear, however, that HTVD is mainly a *microvascular* disease, and that the endothelium is the most extensively involved cell type, somehow leading to vascular hyperpermeability or 'capillary leakage' as the principal pathophysiological finding (Zaki *et al.* 1995). Endothelial susceptibility was showed in laboratory conditions in infant mice infected with HTN (Kurata *et al.* 1983; McKee *et al.* 1985), in bank voles experimentally infected with PUU (Yanagihara *et al.* 1985), and in human umbilical vein endothelial cell cultures inoculated with several HTV strains (Yanagihara and Silverman 1990). Endothelin, a potent vasoconstrictor produced by endothelial cells, may contribute to the transient forms of acute renal failure seen in patients with NE, in whom elevated plasma levels were reported (Forslund *et al.* 1993). Moreover, in the highly lethal hantavirus pulmonary syndrome (HPS), immunohistochemical (IHC) staining showed the presence of hantaviral antigen mainly in the endothelium of lung capillaries, providing another important pathogenetic link (Nichol *et al.* 1993; Zaki *et al.* 1995).

The disease process itself is probably immunological, with a high level of cellular immune reaction, leading to a so-called state of systemic inflammatory response syndrome (SIRS), recently identified by critical care specialists as an early, but often life-threatening reaction to a variety of severe clinical insults (Anonymous 1992). Lymphocytes play a key role, and a decrease (Guang *et al.* 1994) as well as an increase (Nolte *et al.* 1995) of T lymphocytes in the acute phase has been described. The CD4 : CD8 ratio is generally decreased, however, and the presence in the circulation of atypical cells with the aspect of immunoblasts has been considered typical for the recent HPS cases (Hjelle *et al.* 1995*d*; Nolte *et al.* 1995; Zaki *et al.* 1995). There are abundant CD4+ and CD8+ lymphocytes present in the lung interstitium, but there are few polymorphonuclear cells, and there is relatively little necrosis of pneumocytes or other cells as often seen in 'conventional' ARDS (Zaki *et al.* 1995). The lymphocytes may induce migration of macrophages and other inflammatory cells, resulting in the production of cytokines such as tumour necrosis factor-α, (TNF-α), IL-1, IL-2, and γ-interferon, which may in their turn increase vascular permeability. Soluble interleukin-2 receptor (sIL-2 R) levels in sera or plasma of Chinese HTVD patients were in concordance with the degree of illness, i.e. highest during the oliguric phase (Huang *et al.* 1994). Increased levels of pro-inflammatory cytokines have recently been reported in NE cases (Linderholm *et al.* 1995).

We have recently documented very high serum nitrate levels, a measure of nitric oxide (NO), in PUU infected NE patients, showing a high and significant correlation with the Acute Physiological And Chronic Health Evaluation (APACHE II) score and with serum creatinine levels, and an inverse correlation with platelet counts (Groeneveld *et al.* 1995). NO is a highly reactive vasodilatory agent, mainly produced in endothelial cells after stimulation with (amongst other) pro-inflammatory cytokines, and has been implicated in vasodilatation of septic shock (Gomez-Jimenez *et al.* 1995). The highest nitrate levels were found in the two most severe cases, both complicated with ARDS, suggesting a possible role of NO in the, as yet ill-understood, pathogenesis of HPS.

THE HOSTS

THE RODENT VECTOR

In the literature, HTVD is often exclusively mentioned as a rodent-borne disease with strong geographical delineations. In fact, HTVs infect many different animals in nature and have a worldwide distribution. However, it is unknown whether any of the animals other than the natural rodent hosts (Table 30.1) are epidemiologically significant. The correspondence of viral phylogeny and rodent phylogeny argue that the relationship is a close one, and the finding that SNV RNA is less often detectable in antibody-positive animals other than the natural rodent reservoir suggests that persistence may not occur in many of these other infections (Childs *et al.* 1994). In a recent review of available data on this topic, we listed evidence of HTV infection in two different classes of animals (mammals and birds), eight different orders, 24 different families, and a total of 164 different species (Clement *et al.* 1994*c*).

Birds have been described in Russia and Korea (Baek and Lee 1993) as HTV seropositive, and a positive isolation of HTV from a yellow-throated bunting (*Emberizia elegans*) has even been obtained (Slonova *et al.* 1992). Bats (Kim *et al.* 1994) and particularly cats have also been named recently as potential HTV vectors: in 200 feline serum samples from Austria, an IFA seroprevalence for PUU and/or for HTN antibodies of 5 per cent was found (Nowotny 1994). The significance of these findings for human HTVD is still

unclear: to our knowledge, excretion of infectious virus has not been documented yet in these animals. In a recent epidemiological study in The Netherlands, no evidence of PUU antibodies was found in a total of 2025 domestic animals, including 385 dogs and 200 cats (Groen *et al.* 1995). Nevertheless, cat ownership has been described as an epidemiological risk factor for developing HTVD in China (Xu *et al.* 1987). Insectivores such as shrews (Carey *et al.* 1971) and moles (Verhagen *et al.* 1986; Clement *et al.* 1994*c*) can also be infected with HTV, and we registered serologically proven NE cases in Belgium of patients who allegedly became ill after catching a *Neomys fodiens* (water shrew) near a fish pond, or after killing a mole in the garden (unpublished observations).

It is clear, however, that rodents are by far the most important vector for HTV transmission to man (see p. 345). HTVs do not cause apparent illness in these reservoir hosts, which remain infected carriers for life. After experimental PUU infection in bank voles, viruses were detected in the blood, kidneys, liver, urinary bladder, salivary glands, thymus, brown fat, brain, spinal cord, and particularly in the lungs (Gavrilovskaya *et al.* 1990; Yanagihara 1990). The abundance of viral antigen in virtually all organs is found despite an apparently life-long presence of humoral antibodies, also including neutralizing antibodies, as evidenced by the results of plaque reduction neutralization tests (PRNT) (LeDuc *et al.* 1986). This immunological paradox is the hallmark of HTV infection in most, if not all rodent species, and explains the capacity to transmit virus horizontally to other rodents and to man. However, the duration of virus shedding and the period of maximum infectivity remain largely unknown.

HUMANS: ENDPOINT OF AN OTHERWISE INAPPARENT INFECTION

Incubation time is difficult to estimate, since physical contact with rodents is rather an exception than the rule (see p. 338), but is mostly between 4 and 42 days (Zeitz *et al.* 1995). The clinical symptomatology derived from Korean, Russian, and Chinese war literature on HFRS has traditionally been divided in five phases: (1) febrile, (2) hypotensive, (3) oliguric, (4) diuretic, and (5) convalescent (Smadel 1953; Earle 1954). This description is less relevant to the milder European NE forms, and even less so to the severe American HPS forms, but the time framing remains valid for most if not all symptoms:

Febrile phase

Onset is often abrupt and without any prodrome; many patients can situate the exact day and even the moment of the day when symptoms started. Chills, high fever (≥ 39.5 °C), and weakness are often followed by headache, myalgia, and gastrointestinal disturbances (vomiting and/or diarrhoea). Ophthalmological signs are very specific (e.g. not present in flu) but often 'overlooked': acute myopia, blurred vision, photophobia, eye pain, conjunctival injection, and periorbital oedema. Impaired vision in the initial phase can be present in up to 38 per cent of NE cases (Penalba *et al.* 1994) but has never been mentioned so far in American HPS cases. Acute glaucoma can occur, and we encountered two cases of acute bilateral glaucoma presenting in the early phase of NE (unpublished observations). Severe uni- or bilateral lumbar pain due to swelling of the kidney develops 1 or 2 days later. The triad headache + eye pain + lumbar pain is very suggestive and is poorly, or not at all, relieved by analgesics or NSAIDs. The most important laboratory anomaly found in this early phase—and often a clue to diagnosis—is thrombocytopenia (Van Ypersele and Méry 1989; Duchin *et al.* 1994; Penalba *et al.* 1994; Colson *et al.* 1995), the severity of which is often a prognostic sign of complications to come. Conversely, in milder NE forms thrombocytopenia can be so transient as to normalize within 7 days, and can thus be missed (Colson *et al.* 1995).

Hypotensive phase

Hypotension is exceptional in European NE, occasional in severe HTN-induced Asian cases, and usual in American HPS. There is often a rapid progression to marked proteinuria, which can reach levels of 30 g/l (unpublished observations), but is as a rule transitory after a few days. This transient but heavy proteinuria is, together with the cited ophthalmological signs, important in the differential diagnosis with leptospirosis, which can otherwise perfectly mimic HTVD. Episodes of sinus bradycardia despite the feverish condition (Colson *et al.* 1995), and signs of haemoconcentration due to capillary leakage can also be seen in this period.

Oliguric phase

Renal involvement, with a temporary rise in serum creatinine from scarcely pathological levels (90 μmol/l) up to peak levels as high as 1500 μmol/l, can be seen in most Asian series, but seems less frequent in NE, particularly now that more atypical and/or milder cases are recognized by an increased awareness of the physicians. During the 1993 epidemic in the Ardennes, levels of serum creatinine ≥ 150 μmol/l were observed in 75 per cent (40/53) of the Belgian patients (Colson *et al.* 1995), and in only 55 per cent (42/76) of the French patients (Penalba *et al.* 1994). Remission occurs

mostly within 2–3 weeks, and is often heralded by the resolution of thrombocytopenia. Except for a prolonged (several months) loss of urinary concentrating ability, a restoration to normal without sequelae is the rule. Slightly impaired kidney function has sporadically been mentioned as a sequel after severe HTN-like cases in the Balkans (Papadimitriou and Antoniadis 1994) but to our knowledge, irrefutable proof of progression to endstage renal failure has never been documented, i.e. with serial kidney biopsies.

In this stage severe haemorrhagic complications can occur in the Asian HTN-induced forms, but are rare (except for petechiae and/or epistaxis) in NE and uncommon in HPS: 'haemorrhagic' fever without haemorrhages.

Diuretic phase

Diuresis heralds renal, and often general, improvement. Important urinary volumes, however, (up to 12 l/day) can further complicate fluid balance and electrolyte disturbances.

Convalescent phase

This can be prolonged with severe postviral asthenia lasting months. We observed long-standing normocytic anaemia in some NE cases, apparently due to deficient erythropoietin synthesis, which is a product of peritubular tissue in the kidney. Since inflammatory interstital nephritis is the main lesion in NE, this may affect primarily the peritubular region (Clement *et al.* 1993).

Specific features of the hantavirus pulmonary syndrome (HPS)

HPS is characterized by a brief prodromal illness consisting of fever, myalgia, and headache which typically last 3–5 days. Gastrointestinal symptoms are common and, like in PUU virus infections, may simulate a surgical condition. Rapidly progressive, non-cardiogenic pulmonary oedema and severe hypotension (systolic BP $\leq$ 85 mmHg) then follow. With current management, about 40 per cent of patients will die within the first 48 hours of hospitalization from uncorrected hypoxia and/or from intractable shock (Duchin *et al.* 1994; Zaki *et al.* 1995). The syndrome differs clinically from that seen with most ARDS patients, although the distinctive findings may not be present at the time of hospitalization in all cases (Butler and Peters 1994; Ketai *et al.* 1994; Hjelle *et al.* 1995*d*). Clinical haematology values consisting of absolute leucocytosis, relative increase in polymorphonuclear leucocytes, left shift, presence of immunoblasts on smear, and thrombocytopenia are helpful (Hjelle *et al.* 1995*d*; Nolte *et al.* 1995). Pathological findings consist of minimal or moderate interstitial infiltrates of immunoblasts in the lung, congestion and interalveolar oedema (Nolte *et al.* 1995; Zaki *et al.* 1995). SNV antigen is abundant in the endothelium of the lung capillaries, but there is no evidence of a viral cytopathic effect nor viral inclusions (Zaki *et al.* 1995), nor is there a genetic explanation as to why this novel virus seems to attack primarily the lungs (Spiropoulou *et al.* 1994). Where the median value of serum creatinine in the first 17 cases described by Duchin *et al.* (1994) was only of 97.2 μmol/l (range 53.4–221), single cases of HPS in Louisiana and Florida, respectively due to Bayou and BCC viruses, also had acute renal failure (ARF), may be due to the fact that a genetically different (non-SNV) HTV was implicated (see p. 337).

HPS appears to differ consistently from 'classical' HFRS due to PUU and HTN viruses, however. The early period of flushing and conjunctival injection is absent, and the permeability changes are virtually confined to the thoracic cavity in HPS. Mild non-oliguric renal failure is commonly seen in SNV infections, but tubular necrosis and oliguria are not usual. Notably, the first indications of severe disease in HPS are manifest by dyspnoea, hypoxia, and pulmonary infiltrates (Duchin *et al.* 1994; Ketai *et al.* 1994) rather than the renal failure seen in HFRS. However, non-cardiogenic pulmonary oedema and/or full-blown ARDS is not unique to the American forms of HTVD. During the 1993 outbreak of PUU-induced nephropathia epidemica (NE) in the Belgian Ardennes (Clement *et al.* 1994*a*), we described seven patients with varying degrees of non-cardiogenic acute respiratory insufficiency and tachypnoea, indistinguishable from ARDS or its less severe form, now called 'acute lung injury' (ALI) (Clement *et al.* 1994*b*). Heart failure and/or volume expansion could be excluded in all these cases. A similar ALI case during the same NE epidemic was reported from France (Bouly *et al.* 1993). In 1996, two German female patients, both working in the same wool-knitting mill, were described with pulmonary symptoms without kidney involvement. At least one of these presented with all the clinical features suggestive for ALI. In both, nested RT–PCR in serum detected sequences closely related to PUU (Schreiber *et al.* 1996). During the war in Bosnia, a British soldier had to be repatriated after (presumably) a SEO infection. He developed both ARF and severe ALI without signs of fluid overload, prompting intensive ventilation therapy (Stuart *et al.* 1996).

Pulmonary oedema may, of course, be a consequence of volume expansion together with generalized increased capillary permeability, without having the proper (haemodynamic) characteristics of HPS. A majority of the following cases had pulmonary oedema at a time of renal failure, but cardiac and haemodynamic data are often lacking to exclude heart failure:

1. Among severe Chinese forms of HTVD, death in some series has been attributed more frequently to pulmonary oedema (10 fatal cases out of 48) than to haemorrhage (7/48) (Guang *et al.* 1989).
2. The Greek HTN-like isolate *Porogia* virus was obtained from a severely ill soldier with both ARF and ARDS, who had to be ventilated and dialyzed (Antoniadis *et al.* 1987*a*).
3. A German case with PCR products from the urine typical for PUU, was published as a severe non-fatal NE case with lung complications (Pilaski *et al.* 1995). However, the general clinical picture, including generalized oedema, was strongly suggestive for fluid overload, and neither cardiac nor haemodynamic data were given.

DIAGNOSIS

Since most of the clinical signs are atypical, diagnosis should rely on a strong index of suspicion with serological confirmation (Clement *et al.* 1995).

Immunofluorescent assay (IFA) remains for many laboratories the 'gold standard' diagnostic assay for the detection of HTV-specific antibodies. By comparing the titres obtained upon screening with several HTV antigens, IFA can give an indication of the HTV serotype involved in an infection. To obviate the need for P3 biosafety containment, hantaviral antigens can be inactivated by heat (Saluzzo *et al.* 1988), by gamma irradiation (van der Groen *et al.* 1983) or by treatment with β-propionolactone (van der Groen and Elliott 1982). The presence of the HTV-specific antibodies in serum is detected indirectly using a suitable conjugate labelled with fluorescein isothiocyanate (FITC). The assay may be adapted to measure the avidity (functional affinity) of the IgG class of HTV-specific antibodies. It has been reported that this so-called IgG avidity test is up to four times more sensitive than the conventional IgG serology (Hedman *et al.* 1991). Moreover, this test enables an estimation of the moment of infection (whether in humans or rodents) to be made with one single sample. This is possible as low-avidity antibody is generally restricted to the acute or early convalescent phase of illness.

We could also estimate the time of infection with one single serum sample, using enzyme-linked immunosorbent assay (ELISA) for quantification of serum antibodies against group-specific epitopes of HTV glycoproteins and nucleoproteins (Groen *et al.* 1992) and the different classes and subclasses of Ig antibodies formed after infection (Groen *et al.* 1994).

The WHO has recommended that the prototype Hantaan (HTN) virus (isolated from *Apodemus agrarius coreae*, the striped field mouse) and the Puumala (PUU) virus (isolated from *Clethrionomys glareolus*, the bank vole) should be used as routine screening antigens (WHO report, 1991). However, it has been found that the sensitivity of the IFA assay can still be improved upon if a rat-derived Seoul (SEO) HTV strain is included in the battery of IFA screening antigens (Hinrichsen *et al.* 1993; Clement *et al.* 1994*d*; McKenna *et al.* 1994; Stuart *et al.* 1996). In the Americas, a combination of SEO and SNV would be desirable (Feldmann *et al.* 1993; Ksiazek *et al.* 1995).

Since in man (as in rodents) IgG antibodies seem to persist for life, IFA-seropositivity for IgG may not always reflect a recent infection. Moreover, a diagnostic rise in IgG titres has not always been observed in recent clinical cases. Screening for IgM in IFA being less sensitive (Ivanov *et al.* 1988), IgM enzyme immunoassay (EIA) is now generally accepted as the serologic prerequisite for confirming suspected clinical cases. The newer version of this assay, μ-capture EIA, is so sensitive and specific that, when properly performed, it provides an excellent and inexpensive early diagnostic tool (LeDuc *et al.* 1990; Niklasson *et al.* 1990; Ksiazek *et al.* 1995). Similar techniques have been reported to be reliable such as complex-trapping blocking EIA (CTB-EIA) (Groen *et al.* 1989), and μ-capture EIA with recombinant nucleoproteins expressed in *E. coli* (Zöller *et al.* 1991). In the United States, autopsy tissue RNA was reverse-transcribed, amplified by PCR, and ligated to use for expression in *E. coli* to synthesize a recombinant nucleocapsid protein (N) screening antigen for the Western blot and EIA diagnosis of HPS cases, even before the responsible Sin Nombre virus was actually isolated, another major achievement of genetic molecular biology (Feldmann *et al.* 1993; Jenison *et al.* 1994).

The use of recombinant-antigen Western blots with IgG and IgM formats has made rapid clinical diagnosis of HPS possible in the United States and Canada (Hjelle *et al.* 1994*b*, 1995*d*; Jenison *et al.* 1994). In addition to the broadly cross-reactive N protein, the envelope glycoprotein G1 of SNV is recognized by patients with acute SNV-HPS, but G1 exhibits minimal or no cross-reactivity with closely related hantaviruses. Using a series of deletion clones expressing the G1 and N antigens, Jenison and colleagues determined that the predominant linear epitopes of these proteins were localized to 31-amino acid and 59-amino acid domains near the amino-terminus, respectively (Hjelle *et al.* 1994*b*; Jenison *et al.* 1994; Yamada *et al.* 1995). This advance enabled the development of diagnostic tests with higher specificity than available with whole-virus or full-length recombinant N protein diagnostic assays. One especially promising outcome has been the development of a prototype hantavirus recombinant immunoblot assay RIBA™, to be commercialized under the form of a strip, which utilizes both recom-

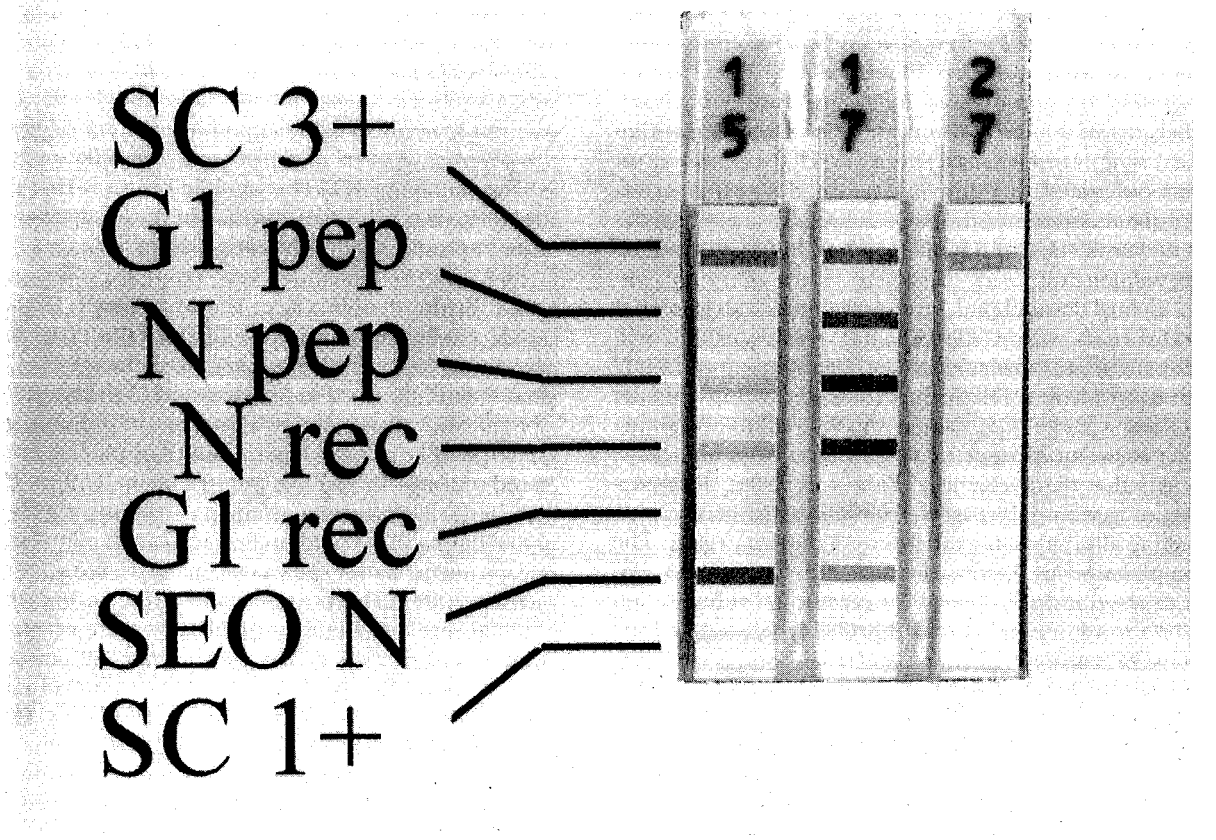

Fig. 30.3 Prototype (for research use only) (Chiron®) RIBA™ hantavirus SIA. Antigens immobilized in bands on the strips are, from top to bottom: (1) a 'serum control' (SC) band in which non-specific IgG antibodies are detected, and arbitrarily rated at 3+ visual intensity; (2) SNV G1 synthetic peptide; (3) SNV N synthetic peptide; (4) SNV N recombinant protein; (5) SNV G1 recombinant protein; (6) SEOV N recombinant protein; (7) a weak serum control (1 + intensity). To be considered positive, a band must be at least as dark as the 1+ serum control band. Strip 15 was probed with serum from a Japanese patient with SEO infection (generously provided by Dr H. W. Lee). Strip 17 was probed with serum from an Arizona patient with acute SNV infection and HPS. Strip 27 was probed with a negative control serum. A 1:50 dilution was used in this 5 hour assay. SNV, Sin Nombre virus; SEOV, Seoul virus; G1, glycoprotein 1; N, nucleocapsid protein; HPS, hantavirus pulmonary syndrome.

binant SNV N and G1 proteins, as well as synthetic peptides that constitute the minimal N and G1 epitopes themselves. This SIA (strip immuno assay) also incorporates recombinant SEO-N protein, to ensure that the assay is sensitive to SEO antibodies as well (Fig. 30.3).

Although the RIBA™ assay is an IgG assay, it has shown considerable promise in the detection of early SNV infection, including the detection of three infections in patients in the prodromal phase of HPS, i.e. before development of lung disease (B. Hjelle, personal communication). As a membrane-based assay that is interpreted visually, it can be used as a rapid (5 hour) diagnostic format in field environments, including settings in which ELISA readers are not available. The high specificity of the G1 antigen for SNV infection in the RIBA™ format has been utilized in the diagnosis of non-SNV HPS cases such as Bayou virus (BAY) in the south-eastern United States (B. Hjelle, personal communication).

The relatively high specificity of G1 antibodies has been confirmed by studies using the radioimmunoprecipitation format (Ravkov *et al.* 1995), which may serve as a reference method for serological typing among related hantaviruses.

The enormous possibilities of PCR genotyping have been discussed already on p. 336. With appropriate primers, this technique allows detection and even genetic classification of viral RNA present in rodent and/or human tissues. However, PCR genotyping still has to prove its value for routine screening of serum or plasma, perhaps due to low HTV titres and/or very transient viraemic phases. RT–PCR performed on freshly frozen tissue is quite sensitive for SNV diagnosis if sera are not available and also provides genetic

information concerning the virus (Nichol *et al.* 1993; Ksiazek *et al.* 1995). Acute blood (mononuclear cells) may also yield a PCR product, although IgM capture serology is more than adequate for routine diagnosis. Because of the low concentrations of RNA in most clinical samples, the sensitivity of the PCR reaction must be enhanced, leading to serious danger of cross-contamination and the need for extreme precautions and verification of the independence of PCR products by sequencing.

Using monoclonal and polyclonal antisera that cross-react with conserved HTV nucleoprotein epitopes, *immunohistochemistry (IHC)*-staining of HTV antigen was possible in formalin-fixed autopsy tissues (Nichol *et al.* 1993; Zaki *et al.* 1995). This technique allows even retrospective diagnosis on stored blocks of cases that died years ago (Zaki *et al.* 1994). Immunostaining with gold particles seems also to be an interesting alternative for the future (Yang *et al.* 1994). The application of RT–PCR to formalin-fixed tissues has been successful but requires careful individualization for a routine procedure and probably offers no advantage in sensitivity (Schwarz *et al.* 1995).

EPIDEMIOLOGY

Until recently, HTVD has been considered a rare disease, with the majority of cases on the Eurasian landmass, and no or only a few case reports from the American and the African continents. On both of these continents, however, evidence existed since many years of HTV infections both in man and in rodents (LeDuc *et al.* 1982, 1984, 1985, 1986; Gonzalez *et al.* 1984; Clement 1987; Yanagihara 1990). However, it is unlikely that the more typical forms of HFRS, particularly when presenting in outbreaks, would have been missed by American and African physicians. It is also possible that many of the HTV infections on these two continents could be subclinical, mild, or atypical (Yanagihara 1990), with the evident exception of the recent American HPS outbreak. Subclinical NE infections are probably the rule in Europe: in a study comparing NE incidence (recorded over 14 years) with IgG IFA PUU antibody prevalence in a highly endemic area of Sweden, it was found that the antibody prevalence rate in the oldest age groups (>60 years) was 14 to 20 times higher than the accumulated life-risk of being hospitalized with NE for men and women, respectively (Niklasson *et al.* 1987). Thus, admissions to hospital are only the top of the iceberg, whereas all other cases are possibly considered as a flu-like viral illness (Kulzer *et al.* 1992) or a viral hepatitis (Yanagihara 1990). This rather reassuring picture was confirmed during sero-epidemiological studies in normal population cohorts in Belgium (Clement and van der Groen 1987) and in The Netherlands (Groen *et al.* 1995), where we found overall positive IgG PUU seroprevalence of 257/21059 (1.3 per cent), and 83/8892 (0.9 per cent), respectively, each time without clinical implications, i.e. the vast majority of IgG seropositives had no suggestive history of renal or other clinical symptoms whatsoever.

The impact of HTVD on public health is best illustrated in two other countries, where the disease has been recognized since the early thirties:

1. In the People's Republic of China, HTVD (or epidemic haemorrhagic fever (EHF) under its local denomination), has been recognized since 1931. The first isolations of *Apodemus*-spread HTN and *Rattus*-spread SEO virus were reported in 1982 (Song *et al.* 1982*a,b*). The accumulated number of officially registered cases in the whole country from 1950 to 1990 reached 904995 with an average morbidity of 2.69 per 100000 inhabitants and an average case fatality rate of 4.3 per cent (range 14.2 per cent in 1969 to 2.1 per cent in 1990). The peak year was 1986, during which 115985 serologically confirmed cases were recorded, with a total of 2561 deaths (fatality rate 2.2 per cent) and a morbidity of 11.08/100000 (Chen and Qiu 1993). From 1988 on, a mean of 40 000 to 50000 annual cases were registered officially (G. Song, personal communication). Although specific serological diagnosis is not routinely practised, it is believed that *Rattus*-spread SEO infections have increased during recent years, even in rural settings, so that nowadays most epidemic areas in the People's Republic of China are of the mixed *Apodemus* and *Rattus* type, i.e. HTN and SEO (WHO Report 1991; Chen and Qui 1993). The age distribution ranges from newborn to 80 years old, but the disease occurs mainly in adolescents and young adults, with a net predominance in males, reflecting probably the agricultural professional exposure, at least for the *Apodemus*-spread infections.

2. In the former USSR, HTVD has been recognized since 1934 and officially registered since 1978. Seroprevalence studies carried out in IFA or direct blocking RIA on a total of 115765 subjects resulted in an overall seropositivity of 3.3 per cent, ranging from 3.5 per cent in the European part to 0.9 per cent in the Far-Eastern part (WHO Report 1991). A total of 68612 cases were registered between 1988 and 1992 (65906 from the European part and 2706 from the Far-Eastern part), with morbidity rates ranging between 1.2 (1982) and 8.0 (1985) per 100000 habitants. The peak year was 1985 with 11413 registered cases. Most patients were in the 20–45-year age group, and males outnumbered females by a ratio of 6:1. Children under the age of 14 years were underrepresented, comprising approximately 5 per cent of the cases. Cases which occurred in the Asian part were

usually caused by viruses similar or identical to HTN virus, and patients frequently experienced severe clinical disease, with case fatality rates of 10–15 per cent.

In the European part, most cases were due to milder infection with PUU-related viruses, with case fatality rates of 1–2 per cent (WHO Weekly Epidemiological Record 1993). The interesting observation that isolates of PUU-like viruses were obtained from two patients of the Asian part of Russia (WHO Weekly Epidemiological Record 1993) were confirmed in Korea (WHO Report 1991), where clinical involvement was noted with a HTV similar to, but not identical with, PUU viruses (H. W. Lee, personal communication). Moreover, PUU-like viruses were recently described in voles (*Cl. rufocanus*) in Hokkaido, Japan (Kariwa *et al.* 1995). So, PUU or PUU-like viruses seem to be present not only in Europe, but in the Far-East as well. Moreover, human infection with the rat-transmitted SEO strain was confirmed serologically among Far-Eastern Russian patients, and by serology and SEO virus isolation in an outbreak of about 50 cases in the European part of Russia (WHO Weekly Epidemiological Record 1993).

It is of interest to compare with the situation in North America. Most diagnosed HTV infections there have been due to SNV or SNV-like viruses and have manifested as HPS. This may be due in part to the severity of HPS and the increased cognisance of the clinical entity. Mild or subclinical SNV infections have been sought and have not been common. Unlike the Eurasian disease, HPS affects both men and women equally, perhaps reflecting the house and surroundings as the principal site of infection (Butler and Peters 1994; Zeitz *et al.* 1995) (Clement *et al.* 1997).

In summary, the picture gets increasingly confused, with different HTV serotypes existing in parallel in the same geographical areas. Moreover, in at least seven recent Belgian cases of ARF, presenting with all the now classical features of nephropathia epidemica (NE) (three patients even presenting with a typical renal biopsy) we were unable to detect at any moment IgG or IgM antibodies against all major HTV serotypes (unpublished data). If confirmed, this leaves the fascinating possibility that we might be confronted with still other related or unrelated viruses, apparently inducing the same clinical picture. Alternatively, PCR signals typical for PUU have been described in two cases which remained seronegative in EIA and in ELISA assays with HTN and PUU antigens (Schreiber *et al.* 1996).

EPIZOOTIOLOGY

HTVD is a zoonosis with a highly specific relationship between each viral serotype and its reservoir species (Table 30.1, Fig. 30.1). In fact, this rodent species, rather than the geographic location, is the most important determinant of the antigenic and genetic profile of a given HTV isolate: e.g. Seoul serotypes from brown rats (*Rattus norvegicus*) all over the world are more closely related to each other than isolates from other rodent species from the same country (Xiao *et al.* 1994; LeDuc *et al.* 1984, 1985; 1986; Schmaljohn *et al.* 1985). This holds true even for the newer isolates such as Dobrava (DOB) (Fig. 30.1). That is, DOB, isolated in Slovenia from *Apodemus flavicollis*, subfamily Murinae of the Muridae family, is genetically close to the other *Apodemus* isolates from Asia, such as HTN. THAI, isolated from a *Bandicota* related to *Rattus*, falls in the same clade as other viruses carried by murid rodents, subfamily Murinae, but is particularly similar to *Rattus*-isolates i.e. SEO-like viruses (Xiao *et al.* 1994) (Fig. 30.1).

However, this strict host–virus relationship was put into question by a report of isolation of a PUU-like virus called POZ-M1, from house mice (*Mus musculus*) captured in and around habitats of patients with a severe form of HTVD in Serbia (Diglisic *et al.* 1994). Moreover, another PUU-like virus without relation to human disease, had already been isolated in Leakey, Texas, USA from *Mus musculus* (Baek *et al.* 1988). There is growing suspicion that this Leaky isolate was not independent (Xiao *et al.* 1994). Additional confirmation is needed to demonstrate that *Mus* sp., including laboratory mice, might harbour pathogenic HTV. Regardless, there is a hazard for laboratory personnel in handling laboratory rats that have not been properly screened and maintained free of hantaviruses (Mc Kenna *et al.* 1992).

Despite this possible exception, it can be maintained that the four clinically most important HTV strains are Hantaan (HTN), Seoul (SEO), Puumala (PUU), and Sin Nombre virus (SNV), respectively spread by *Apodemus agrarius, Rattus norvegicus, Clethrionomys glareolus*, and *Peromyscus maniculatus* (Table 30.1). So far, HTVs carried by other murid rodent species of the same subfamily always appeared to be closely related to each other. SEO is the only serotype with a worldwide distribution and an ever growing importance, perhaps due to the fact that *Rattus* sp. and their associated viruses have been transported for many years throughout the world on cargo ships (LeDuc *et al.* 1984, 1986) (Clement *et al.* 1997).

In the People's Republic of China, HTV isolates and/or HTV antigen were detected in 55 species of Vertebrata including 37 species of Rodentia; the field mouse (*Apodemus agrarius*) and the Norway rat (*Rattus norvegicus*) being the most important vectors, with 5.3 per cent (3497/65824) and 4.9 per cent (3789/77295) positivity, respectively. *Apodemus agrarius* was confirmed as most frequently present in rural areas (53.7 per cent

of all captured rodents), versus *Rattus norvegicus* in residential areas (52.1 per cent) (Chen and Qiu 1993).

In the former USSR, a total of 300000 small mammals belonging to 63 species were collected from all ecological zones, and 45 species were found to be antigen positive (WHO, Weekly Epidemiological Record 1993). HTN in the Far-Eastern part was mainly found in *Apodemus agrarius*, whereas PUU in European and Siberian areas was mainly spread by *Clethrionomys glareolus*.

In Europe, the red bank vole (*Clethrionomys glareolus*) was shown by several authors to be the most important rodent vector for NE (Niklasson and LeDuc 1984; Groen *et al.* 1995; Verhagen *et al.* 1986). We confirmed these findings during a zoosurvey set up in Belgium, The Netherlands, and Germany between 1986 and 1990, during which 5038 wild small mammals were captured. Of a total of 2225 animals examined, 153 (6.88 per cent) showed (by IFA or ELISA) the presence of a PUU-like antigen in the lungs, whereas 194 (5.22 per cent) of a total of 3718 examined showed the presence of IFA IgG hantaviral antibodies in the serum. *Clethrionomys glareolus* was the second most abundant (total number captured, 2012), and in each country by far the most infected rodent species, showing antigen in 14.5 per cent and antibody in 13.3 per cent of the examined animals. However, significantly higher PUU-like antigen presence was found in the lungs of *Cl. glareolus* in Chimay (Belgium, 1986) with 35.5 per cent (11/31), in Enschede (The Netherlands, 1989) with 40 per cent (4/10), and in Ulm (Germany, 1990) with 22.9 per cent (8/35). In all these localities, a cluster of human NE had been noted shortly before (Clement *et al.* 1992, 1996).

A much more complex picture is seen in the Balkans (Table 30.1), where at least four clinically important HTV strains have been reported, each with their respective rodent vector: PUU spread by *Clethrionomys*, and possibly also by *Mus* (Diglisic *et al.* 1994), HTN or HTN-like spread by *Apodemus* (Antionadis *et al.* 1987*b*; Gligic *et al.* 1989; Avsic-Zupanc *et al.* 1994) and DOB by *Apodemus* (Avsic-Zupanc *et al.* 1992). Moreover, we have reported SEO-induced acute human disease in a Canadian UN soldier in 1992 in Sarajevo (Bosnia) after a clear history of rat exposure (Clement *et al.* 1994*d*). This was the first PRNT-proven evidence of SEO-infection in Europe, followed by another SEO-suspected case from the same region in a British soldier (Stuart *et al.* 1996). With these, the rat can be confirmed as an underestimated vector for spreading less benign forms of HTVD, not only in the laboratory (Desmyter *et al.* 1983; Mc Kenna *et al.* 1992), but also in urban and rural settings. We have described the first 16 acute cases of HTVD in Northern Ireland, a region where *Cl. glareolus* is not present, all reacting in IFA to R22, a rat-borne SEO serotype (McKenna *et al.* 1994).

Since 1982, wild rats have been described as HTV reservoirs (SEO-serotype) in ports and other cities in the United States, showing IgG IFA prevalences as high as 74 per cent in some alleys of Baltimore (LeDuc *et al.* 1982; Yanagihara 1990). However, only recently have mild SEO-induced human cases of HTVD been described in the United States (Glass *et al.* 1994). Moreover, in a study conducted in 8080 Baltimore city residents, an intriguing epidemiological association was found between seropositivity for a local SEO-strain called 'Baltimore rat virus', and hypertensive chronic renal disease, apparently unrelated to other renal disease (Glass *et al.* 1993).

Rats in South America have also been described as heavily infected with a SEO serotype, with prevalence rates up to 56 per cent in Belem (Brazil) (LeDuc *et al.* 1985). Already in 1990 we found in 8 (5.1 per cent) out of 156 Brazilian patients, first suspected of having leptospirosis, serological evidence of a SEO infection (Hinrichsen *et al.* 1993). In retrospect, these appeared to be the first reported cases of serologically proven HTV infection on the American continent.

Another important rodent vector in North America is the meadow vole (*Microtus pennsylvanicus*) carrying the Prospect Hill (PH) serotype (Table 30.1). No human disease has been ascribed to PH so far, despite the fact that PH-seropositive mammalogists were found, some of them with a history of non-A non-B hepatitis (Yanagihara 1990).

A major breakthrough in the understanding of the role of rodents in HTV transmission came after the May 1993 outbreak of hantavirus pulmonary syndrome (HPS) in the south-western United States. Probably due to a disturbance of the local ecological balance (heavy rains and snow during the previous spring, after a long drought), an abundance of rodent food, including piñon nuts and grasshoppers, suddenly appeared. The increased forage, and perhaps other factors, resulted in a marked population increase in the deer mouse (*Peromyscus maniculatus*), a sigmodontine rodent of the Muridae family and one of the most common mammals in North America. Between May 1992 and May 1993, deer mouse numbers were estimated as tenfold above usual (Stone, 1993), particularly in the semiarid regions of the states New Mexico, Arizona, and Colorado. Thirty per cent of the *P. maniculatus* trapped in or around habitats of proven HPS cases appeared to be seropositive to (cross-reacting) Prospect Hill (PH) (Childs *et al.* 1994; Ksiazek *et al.* 1995). Interestingly, all newer non-SNV viruses inducing HPS, such as NYV, BCC, and BAY, are also harboured in New World species of the subfamily Sigmodontinae, family Muridae, whereas all Old World HTV are hardboured by species of the subfamily Arvicolinae (*Clethrionomys* sp., *Microtus* sp.) or subfamily Murinae

(*Rattus* sp., *Apodemous* sp), suggesting again that HTV and their predominant rodent hosts have co-evolved for a very long time (Butler and Peters 1994) (Hjelle *et al.* 1995*d*).

TREATMENT, PREVENTION, AND CONTROL

THERAPY

As in so many other viral diseases, therapeutic possibilities are limited. Supportive medicine may be sufficient in the majority of cases, since self-remittance is the rule, at least in the milder European NE cases. For the flu-like symptoms, a safe analgesic such as paracetamol should be preferred over the more potent non-steroidal anti-inflammatory drugs (NSAID), in view of the potentially deleterious effect of the latter on kidney function. A further advantage of prescribing paracetamol is the fact that this drug does not enhance the risk of haemorrhagic complications. In severe HTV cases, acute dialysis for one or several sessions may be indicated, often for regulating fluid overload after prolonged oliguria. In our Belgian NE series (Colson *et al.* 1995), as in most other European series (Lähdevirta *et al.* 1984; Settergren *et al.* 1988; Van Ypersele and Méry 1989), artificial kidney treatment was necessary in 1–5 per cent of the cases. In Greece, however, the clinical picture due to a HTN-like virus Porogia virus was more severe, prompting dialysis in 30 per cent (41/138) of the patients (Papadimitriou and Antoniadis 1994).

Mechanical ventilation was often indicated as supportive treatment for the respiratory insufficiency characteristic or the American HPS cases (Duchin *et al.* 1994;) (Hjelle *et al.* 1995*d*), but appeared also to be necessary in severe European cases complicated with pulmonary oedema, induced by HTN-like serotypes (Antoniadis *et al.* 1987*a*), SEO cases (Stuart *et al.* 1996), and even by the 'benign' PUU serotype (Clement *et al.* 1994*a,b* Pilaski *et al.* 1995). Since in the latter cases, both lungs and kidneys may be affected, and haemorrhagic complications are often present, we are confronted with extremely ill patients needing the full array of intensive care medicine. The same might also apply to patients infected with the recently isolated Dobrava strain, having a reported fatal outcome of 20 per cent (Gligic *et al.* 1992).

DRUG TREATMENT

The antiviral drug ribavirin (a nucleoside analogue), when given in early treatment, has proven beneficial for the therapy of severe Far-Eastern cases (Huggins *et al.* 1991). Ribavirin therapy of HPS in an open-label trial gave inconclusive results, resulting in the organization of a randomized, controlled trial. The drug seems less suited for the mostly mild PUU-induced cases.

VACCINES

As for most other viral diseases, the final answer to HTVD will be prevention by means of a safe, cheap, and generally applicable (i.e. worldwide) vaccine, a goal that has not yet been reached. However, the possibilities for priming for immunopathology exist (Yao *et al.* 1992). Target populations, in which attack rates and disease severity are sufficiently high to justify vaccination, have not been clearly defined, although areas of Asia with high HTN virus transmission and no likelihood of effective rodent control, would certainly be candidates. In the Republic of Korea a vaccine, based on a formalin-inactivated HTN virus (ROK 84–105), derived from infected suckling mouse brains, was issued (WHO Report 1991), but is awaiting further field trials. In China, field trials are under way with inactivated vaccines from golden hamster (*Mesocricetus auratis*) kidney cell cultures (GHKC), suckling mouse brains (MB), and Mongolian gerbil (*Meriones unguiculatus*) kidney cell (MGK) cultures (WHO Report 1991). Encouraging preliminary results with up to 93.2 per cent seroconversion rates have been reported (G. Song *et al.* 1991). Finally, C. Schmaljohn and coworkers, in the United States, developed a vaccinia-vectored recombinant vaccine against HTN, giving excellent humoral and cell-mediated immune responses in preclinical and phase I clinical trials (Schmaljohn *et al.* 1995*b*). However, all these vaccines are based upon only one serotype (HTN), or at best on both HTN and SEO (G. Song *et al.* 1991), which may be a problem for a full protection in view of the (ever-growing) list of clinically important hantaviral strains (Table 30.1, Fig. 30.1). It may be necessary to prepare vaccines against multiple strains, although cross-protection may be useful among more closely related viruses. The situation is best exemplified in Bosnia, where soldiers living under war conditions would have been the first to benefit from a plurivalent vaccine (Clement *et al.* 1994*d*, 1996; Stuart *et al.* 1996). To broaden the efficacy of the vaccine, and to increase safety, the use of alternate pox-viruses as recombinant vaccine vectors for both HTN and PUU viruses are now under investigation (Schmaljohn *et al.* 1995*b*).

RODENT CONTROL MEASURES

So far, China seems to be the only country where control measures and particularly large-scale rat extermination programmes may have curved the epidemiological figures of HTVD cases downwards, at least those

induced by the rat-transmitted SEO serotype (WHO Report, 1991). For recently proven highly endemic areas (i.e. foci with proven recent human cases and a proven recent high infection rate in the local rodent population), it may be indicated to discourage intensive outdoor activities such as camping, caravaning, digging, and particularly low-crawl training (e.g. during military manoeuvres), at least in areas with numerous rodent burrows (Clement *et al.* 1996). For the 1993 HPS epidemic in the United States, nationwide guidelines on leaflets, TV and even on video cassettes were distributed and focused on the removal of rodent shelters and food sources in and around houses, eliminating rodents inside houses, and limiting access of rodents to human habitats (MMWR 1993*a*). These recommendations were based on data showing increased numbers of infected rodents in case homes and other indications suggesting that infection occurred in or around households (Zeitz *et al.* 1995). Studies are now under way to evaluate the impact of these measures.

In a recent case-control study of HPS integrating rodent trapping data, not only agricultural activities such as hand-plowing appeared to be risks, but also entering and cleaning closed buildings such as food storage areas and animal sheds (Kopela and Lähdevirta 1978; Zeitz *et al.* 1995). Even exercising in a rat-infested building can be a risky activity (Clement *et al.* 1994*d*). Disturbing rodents results in the shedding of urine, and cleaning activities may produce secondary aerosols from recent excreta.

Probably such structures should be opened for a period and rodents eliminated, before cleaning using appropriate wetting and disinfection, e.g. with a dilute hypochlorite solution (MMWR 1993*a*; Zeitz *et al.* 1995).

It has proved feasible to eradicate HTVs from infected laboratory rat colonies by applying Caesarean section and foster mother techniques. This approach enables the transmission of the virus to be curbed, even under laboratory breeding and maintenance conditions (McKenna *et al.* 1992).

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