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Hantavirus infection in East Asia

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Abstract

Hantaviruses are enveloped RNA viruses that belong to the *Hantavirus* genus of the family *Bunyaviridae*. These viruses persistently infect their rodent reservoirs without causing disease. The virus is transmitted to humans via the inhalation of infectious aerosols generated from contaminated animal secretions or through the contaminated saliva of animal bites. Hantaviruses cause haemorrhagic fever with renal syndrome in Euro-Asia, and hantavirus pulmonary syndrome (HPS) in North and South America. Here, we review the epidemiology and epizootiology of hantavirus infection in Asian countries.

Keywords: Zoonosis; Bunyavirus; Rodent; HFRS; HPS; Renal; Pulmonary; Persistent infection

Résumé

L'Hantavirus est un virus, enveloppé d'ARN, classé comme une espèce de Hantavirus de la famille Bunyaviridae. Ce virus infecte constamment des réservoirs rongeurs sans provoquer immédiatement la maladie. Le virus est transmis aux humains par l'intermédiaire de l'inhalation d'aérosols infectieux, qui sont fabriqués des sécrétions animales contaminées, ou par l'intermédiaire de la salive contaminée des animaux lorsque les humains sont mordus par un animal. La fièvre hémorragique sera provoquée par l'infection à Hantavirus et cette

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fièvre est souvent accompagnée du syndrome rénal (HFRS) dans les régions Euro-Asies, tandis qu'elle provoque le syndrome pulmonaire à Hantavirus (SPH) dans l'Amérique du Nord et du Sud. Dans ce document, nous révisons l'épidémiologie et l'épizootiologie de l'infection à Hantavirus dans les pays asiatiques.

Mots clés: Zoonose; Bunyavirus; Rongeur; HFRS; HPS; Renal; Pulmonaire; Persistant infection

1. Introduction

Hantaviruses form a separate genus, *Hantavirus*, within the family *Bunyaviridae*. To date, 22 *Hantavirus* species have been registered within this genus based on comparisons of nucleotide sequence similarity and evolutionary classifications of viral genomes [1].

Hantavirus infection includes two different forms of severe febrile diseases, haemorrhagic fever with renal syndrome (HFRS) [2] and hantavirus pulmonary syndrome (HPS) [3]. The virus is maintained in persistently infected rodents, which therefore serve as natural viral reservoirs. Transmission of the virus to humans and rodents occurs via the inhalation of infectious aerosols generated from hantavirus-containing animal secretions [4–6] or by the contaminated saliva of animal bites [7]. Other viruses in the *Bunyaviridae* are transmitted by arthropod vectors [8].

Each *Hantavirus* species is predominantly carried by one species of rodent [9]. Phylogenetic analysis of the hantavirus genome has demonstrated three distinct clades, each composed of viruses isolated from rodent hosts belonging to the same subfamily. Thus, viral clades for the subfamilies *Murinae* (Old World rats and mice), *Arvicolinae* (voles and lemmings of the Northern Hemisphere) and *Sigmodontinae* (New World mice and rats) have been identified [10]. Thottapalayam virus (TPMV) is the only hantavirus isolated from a non-rodent host, the house musk shrew *Suncus murinus*, which was first captured in southern India in 1964 [11]. The phylogeny of the hantaviruses, including TPMV, has been shown to mirror the genealogical relatedness of their host animals, suggesting their co-evolution [12,13].

Due to the close relationship between hantavirus and rodent species, the distribution of cases of HFRS and HPS has been confined to the geographic distributions of the viral host species. Thus, since the reservoir animal species for the virus that causes HPS inhabits North and South American countries, the disease has only been reported in those regions [10]. Similarly, reservoir animals for Hantaan (HTNV) [14], Dobrava (DOBV) [15,16], and Puumala (PUUV) [17] viruses, which cause HFRS, live primarily in eastern Asia, northern and eastern Europe, central Europe, and central to northern Europe, respectively, and cases of disease caused by infections with these viruses are confined to the corresponding region. However, the HFRS-causing Seoul virus (SEOV) [18] is found worldwide, probably due to the distribution of its infected host, the brown rat, through international freight transportation. Nonetheless, HFRS resulting from SEOV infection has been confined, thus far, to Asian countries [19].

The total number of HFRS patients is about 60,000–150,000 annually. More than 90% of these cases occur in Asian countries, including China, Russia, and Korea. Epidemiologic and epizootiologic information regarding the incidence of hantavirus infection in other East Asian countries is still limited, although patients with fevers of unknown etiology are suspected of being infected with hantavirus [20].

HPS was first recognised in the southwestern United States (US) in 1993 as an acute respiratory distress syndrome (ARDS) with greater than 40% mortality [3]. Between the first case in 1993 and June 2006, 451 cases (159 deaths) in 32 states in the US [21] and 62 cases in Canada [22] have been reported. In South American countries, 1685 cases resulting in 331 deaths were recorded from 1993 to 2004 in Panama, Brazil, Bolivia, Chile, Paraguay, Uruguay, and Argentina [23]. The numerous cases of hantavirus and HPS in North and South America confirm the importance of this rodent-borne zoonosis in the New World (Table 1).

In this report, we briefly review current knowledge of hantavirus and hantavirus-mediated diseases, and then discuss hantavirus infection in humans and rodents in Asia. Finally, we assess the potential threat of an outbreak of hantavirus infection in Asian countries.

2. Hantavirus

Hantaviruses are enveloped, negative-sense RNA viruses [2]. The hantaviral particle is spherical or oval in shape with a diameter ranging from 80 to 120 nm. The genome of the virus consists of small (S), medium (M) and large (L) segments, 1696–2059, 3616–3696 and 6530–6562 nucleotides long, respectively [24]. The S, M and L genome segments encode nucleocapsid protein (N, 48–54 kDa), two external glycoproteins [Gn (68–76 kDa) and Gc (52–58 kDa)], and transcriptase protein (L, 246–247 kDa), respectively [25]. Unlike other members of the *Bunyaviridae*, hantaviruses do not encode non-structural proteins [24]. Hantavirus virions are formed on membranes of the host-cell Golgi complex, followed by budding into Golgi cisternae [26] and release of the virions by exocytosis.

Thus far, 22 virus species have been assigned to the *Hantavirus* genus based on the criteria of more than 7% difference in amino-acid identities of the complete glycoprotein precursor and N sequences, and a more than fourfold difference in two-way cross-neutralisation tests involving species of primary reservoir animals [1].

3. Diseases caused by hantavirus infection

3.1. Hantavirus diseases in humans

In the prodromal phase, HFRS and HPS produce similar flu-like symptoms, such as high fever, myalgia and headache. Subsequently, however, the symptoms associated with HFRS [27] and HPS [28] are very different.

HFRS is characterised by systemic involvement of the capillaries and small vessels, which causes capillary leakage and haemorrhagic manifestations. Renal involvement in the form of acute renal dysfunction as a result of interstitial haemorrhage and

Table 1
List of Old World and New World Hantaviruses

Virus species	Strain	Abbreviation	Principal species	Original isolation in	Geographical distribution of reservoir	Disease
Old World Hantaviruses						
Murinae subfamily-associated viruses						
Hantaan virus	Amur virus	AMRV	*Apodemus peninsulae*	Far East Russia	East Asia	HFRS
	Da Bie Shan virus	DBSV	*Niviventer confucianus*	South East China	East Asia	?
	Hantaan virus-76-118	HTNV	*Apodemus agrarius*	Korea	East Asia	HFRS
Dobrava-Belgrade virus	Dobrava-Belgrade virus	DOBV	*Apodemus flavicollis*	Slovenia/Servia	East Europe	HFRS
	Saaremaa virus	SAAV	*Apodemus agrarius*	Estonia	East Europe	HFRS
Seoul virus	Seoul virus-HR80-39	SEOV	*Rattus norvegicus*, *R. rattus*	Korea	Worldwide	HFRS
	Seoul virus-L99	SEOV	*R. losea*	Korea	Worldwide	HFRS
	Seoul virus-SR-11	SEOV	*R. norvegicus* (laboratory rat)	Japan	Worldwide	HFRS
Thailand virus	Thailand virus	THAIV	*Bandicota indica*	Thailand	South East Asia	?
Arvicolinae subfamily-associated viruses						
Puumala virus	Hokkaido virus-Kamiiso-8Cr-95	PUUV	*Clethrionomys rufocanus*	Japan	East Asia	?
	Muju virus	MUJV	*Eothenomys regulus*	Korea		?
	Puumala virus-Sotkamo	PUUV	*Clethrionomys glareolus*	Finland	Northern Europe	HFRS
Khabarovsk virus	Khabarovsk virus	KHAV	*Microtus fortis*	Far East Russia		?
Tula virus	Tula virus-Tula/Ma76/87	TULV	*Micotus arvalis*, *M. rossiaemeridionalis*			?
	Tula virus-Moravia/Ma530	TULV	*Micotus arvalis*, *M. rossiaemeridionalis*		?	
Topografov virus	Topografov virus	TOPV	*Lemmus sibiricus*			?
Not defined (suggested from *Insectivor*-associated virus)						
Thottapalayam virus	Thottapalayam virus	TPMV	*Suncus murinus*	India	East Asia?	?

Table 1 (*continued*)

Virus species	Strain	Abbreviation	Principal species	Original isolation in	Geographical distribution of reservoir	Disease
New World Hantaviruses						
Sigmodontinae subfamily-associated viruses						
Andes virus	Andes virus	ANDV	*Oligoryzomys longicaudatus*	Chile, Argentina	South America	HPS
	Bermejo virus	BMJV	*Oligoryzomys chacoensis*			
	Lechiguanas virus	LECV	*Oligoryzomys flavescens*			
	Maciel virus	MCLV	*Bolomys obscurus*			
	Oran virus	ORNV	*Oligoryzomys longicaudatus*			
	Pergamino virus	PRGV	*Akadon azarae*			
Bayou virus	Bayou virus	BAYV	*Oryzomys palustris*	Eastern USA	North America	HPS
Black Creek Canal virus	Black Creek Canal virus	BCCV	*Sigmodon hispidus*	South Eastern USA	South East USA, Northern South America	HPS
Cano Delgadito virus	Cano Delgadito virus	CADV	*Sigmodon alstoni*	Venezuela		?
El Moro Canyon virus	El Moro Canyon virus-RM-97	ELMCV	*Reithrodontomys megalotis*		Canada, USA, Mexico	?
Laguna Negra virus	Laguna Negra virus	LANV	*Calomys laucha*	Paraguay, Bolivia		HPS
Muleshoe virus	Muleshoe virus	MULV	*Sigmodon hispidus*	South East USA, Northern South America		?
New York virus	New York virus-RI-1	NYV	*Peromyscus leucopus*	USA, Canada, Mexico		HPS
Rio Mamore virus	Rio Mamore virus	RIOMV	*Oligoryzomys microtis*	South America		?
Rio Segundo virus	Rio Segundo virus	RIOS	*Reithrodontomys mexicanus*	Central America		?
Sin Nombre virus	Blue River virus-Indiana	BRV	*Pelomyscus leucopus*	Central USA		HPS
	Blue River virus-Okahoma	BRV	*Pelomyscus leucopus*			
	Monongahera virus	MGLV	*Pelomyscus maniculatus*	Eastern USA, Canada		
	Sin Nombre virus-Convict Creek 107	SNV				

Table 1 (*continued*)

Virus species	Strain	Abbreviation	Principal species	Original isolation in	Geographical distribution of reservoir	Disease
	Sin Nombre virus-NMH10 virus	SNV	*Peromyscus maniculatus*	US		
Arvicolinae subfamily-associated viruses						
Prospect Hill virus	Bloodland Lake virus	BLLV	*Microtus ochrogaster*	USA, Canada		?
	Prospect Hill virus	PHV	*Microtus pennsylvanicus*	USA, Canada		
Isla Vista virus		ISLAV	*Microtus californicus*	Western USA		?

interstitial infiltrates is also common. After the prodromal period, the clinical course of patients with severe disease can be divided into five phases: febrile, hypotensive, oliguric, diuretic and convalescent [29]. A more mild type of hantavirus infection, nephropathia epidemica (NE), is caused by PUUV and occurs in northern Europe [30]. In NE patients, although renal manifestations are common, haemorrhage is rare and the five phases typical of severe HFRS are absent. The mortality of NE patients is 0.1–0.3% and is thus much lower than the 5–10% of HFRS patients infected with HTN, SEO or DOB viruses [9].

HPS is characterised by bilateral interstitial pulmonary infiltrates, respiratory compromise usually requiring the administration of supplemental oxygen and clinical symptoms resembling those of ARDS. HPS can be divided into two phases: a prodromal phase, which usually lasts 3–5 days, and a cardiopulmonary stage marked by diffuse pulmonary oedema and hypotension within 2–5 days after the onset of pulmonary symptoms. The rapid progression of interstitial pulmonary oedema to alveolar oedema, with severe bilateral involvement and the accumulation of pleural effusion, accounts for the 30–40% mortality associated with HPS [29].

Although the characteristic symptoms of HFRS and HPS differ, increased capillary permeability is considered to be the common underlying factor of the two diseases [31,32]. Since hantavirus is usually non-cytopathogenic in cultured cells, cell-mediated immune responses, such as activation of virus-specific CD8+ T cells and increased levels of tumour necrosis factor receptor (TNF-r), interleukin (IL-6 and IL-10) are most likely responsible for the symptoms observed in HFRS and HPS [33].

3.2. Disease in animals

All hantavirus reservoir animals are rodents, with the exception of TPMV [11], whose reservoir is *S. murinus*, a member of the order Insectivore. As is the case for

many other zoonoses, all natural reservoirs of hantavirus are asymptomatic following infection, and in many of these animals, a persistent infection is established, although the animals possess high titres of neutralising antibody [18]. The suppression of cellular immunity has been considered as the most plausible mechanism behind the maintenance of persistent infection [34] but this has yet to be determined conclusively due to the technical difficulties in immunologically characterising wild rodents. However, hamsters infected with the Andes virus, which belongs to the genus *Hantavirus*, manifest symptoms similar to those associated with HPS, such as pulmonary oedema, accumulation of pleural exudates and high mortality [35]. Therefore, the hamster model has provided a useful tool for studying the mechanisms of HPS pathogenesis.

Although few reports exist on hantavirus infection of *Mus musculus* in nature, laboratory mice are highly susceptible [36]. In particular, newborn mice [37] and immunologically defective animals, such as nude mice [38] and SCID mice [39,40], were found to be vulnerable to systemic infection with a fatal outcome, whilst surviving mice became persistently infected, with suppression of the virus-specific CD8 T-cell response [34]. This observation supports the suppression of cellular immunity as the mechanism establishing persistent infection of hantavirus reservoir animals.

4. Epidemiology and epizootiology of HFRS in Asian countries

Since more than 90% of HFRS cases have been reported in eastern Asia, including China, the far-eastern parts of Russia, and Korea, epidemiological and epizootiological studies have mainly been conducted in those countries. Accordingly, this section will first review the results of those studies and then summarise what is known about hantavirus infection in other East Asian countries, particularly in Southeast Asia.

4.1. East Asia

The majority of reported cases of hantavirus infection in East Asia have come from China [41]. HFRS was first recognised in northeastern China in 1931 as a febrile disease with haemorrhagic manifestation and renal dysfunction. Beginning in 1955, the areas in which the disease was endemic expanded to many other parts of the country. The number of cases of HFRS in China from 1950 to 1997 was 1,258,402. From 1990 to 1997, the number of cases, morbidity (per 100,000) and number of deaths (fatality %) were 391,046 (4.21%) and 6310 (1.61%), respectively. More recent reports examined the distribution of HFRS cases for all of mainland China [42]. From 1994 to 1998, 257,127 cases of HFRS were reported; these were documented in 28 of 31 provinces and in 1397 of the country's 2359 counties. However, spatial analysis indicated hot spots, i.e., large ($>10{,}000\,km^2$) areas where the risk of HFRS risk was higher, in Shandon, Hebei, Heilongjiang, Hunan, Zhejiang, Jiangxi and Guangxi provinces. Furthermore, in several smaller areas ($<10{,}000\,km^2$) the risk of HFRS was also found to be higher. These areas were

located in provinces of central, eastern and northeastern China. Therefore, as an infectious disease, HFRS is an important public health concern throughout China.

A nationwide study of the epidemiology of HFRS conducted between 1984 and 1986 indicated that 67 species of vertebrates were infected with hantaviruses. Most of those animals were rodents, but several species of domestic animals, including cats, pigs, rabbits and dogs were reported [41]. Antigenic and genetic characterisations of the hantaviruses isolated from HFRS patients and from rodents in China suggested that two different hantaviruses, HTNV and SEOV, and viruses related to them, were co-circulating in endemic areas [43]. Phylogenetic analysis based on the nucleotide sequences of the S and M genome segments of 46 hantaviruses from China, 13 from patients, 23 from rodents and 10 from unknown hosts, were compared with hantaviruses from Korea, Japan, the US and Europe [44]. The HTNV type was divided into nine distinct genetic subtypes, one consisting of isolates from Korea. The Da Bie Shan virus strain NC167 of HTNV, isolated from a Chinese white-bellied rat (*Niviventer confucianus*) captured in a mountainous area of Anhui Province, was the most distant from the remaining HTNV strains. A comparison of the M segment of HTNV with those of eight other genotypes revealed 24.1–25.0% nucleotide and 15.3–16.2% amino-acid sequence differences. By contrast, the nucleotide and deduced amino-acid sequence differences in the M segments of the remaining HTNV strains were 5.4–15.4% and 1.8–4.6%, respectively. Strain Da Bie Shan NC167 and HTNV strain 76118, which is a prototype of HTNV, also showed more than fourfold two-way differences in cross-neutralisation tests. Based on these findings, strain Da Bie Shan NC167 was classified as a novel type of hantavirus [44] (Fig. 1).

A correlation between the subtype and province of origin was established for seven of the nine genetic subtypes of HTNV, which suggests immigration of the reservoir rodent of ancestral HTNV from the southern province of Guizho to three northern provinces (Helongjian, Shandong and Zhejiang) and to Korea. SEOV is also divided into five genetic subtypes, but the variability amongst them is smaller than that between HTNV subtypes. Of those five subtypes, the Gou3 virus, isolated from *Rattus rattus*, showed the greatest divergence from the other four SEOV subtypes, which were isolated from *Rattus norvegicus*. The diversity in the M segment nucleotide sequence and the Gn/Gc amino-acid sequence between Gou3 and the other SEOVs was 15.4–16.0% and 3.1–3.6%, respectively, compared to 3.5–1.9% and 0.5–1.1%, amongst the other SEOVs [44].

Far-eastern Russia is also an area where hantaviruses are endemic. The first case of HFRS in Asian Russia was reported in 1934, in the Khabarovsk region [45]. Since then, many other cases of HFRS have been reported throughout Russia. Although the majority of cases (84,687) reported between 1978 and 1995 occurred in the European part of the country, several different hantaviruses are known to be present in far-eastern Russia and to cause HFRS. In fact, from 1978 to 1995, 3145 cases were registered in 15 of the 29 regions of Asian Russia [46].

In northeastern China, HTNV carried by *Apodemus agrarius* caused several severe cases of HFRS. PUUV infection has been found amongst *Clethrionomys rufocanus* in Asian Russia and may be the cause of a milder form of HFRS. Recently, Amur virus

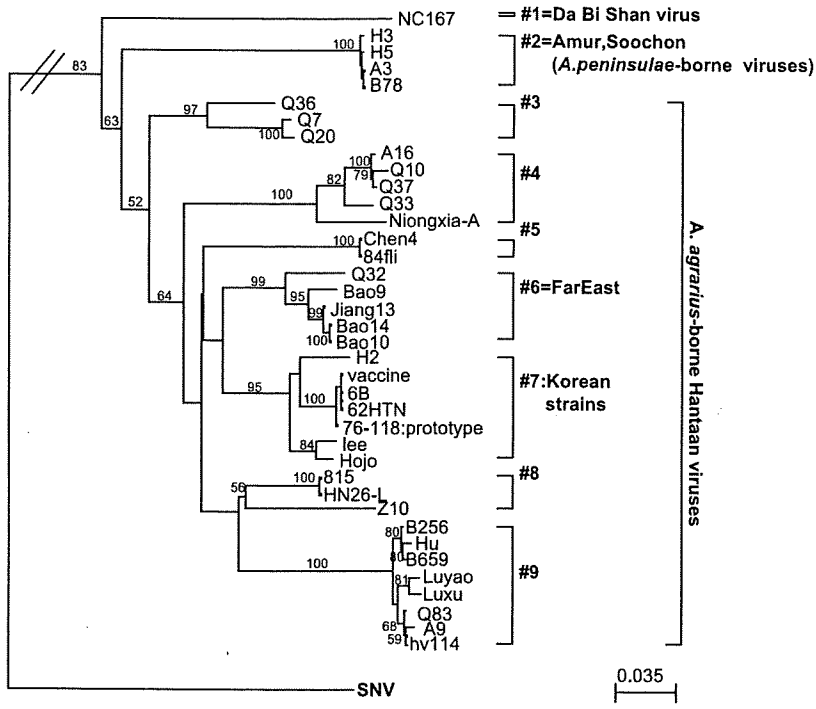

Fig. 1. Phylogenetic tree for *Apodemus*-borne hantaviruses. The neighbour-joining phylogenetic analysis was performed based on the partial sequences of the M (nucleotides 2001–2301) segment. The numbers at the nodes are bootstrap confidence levels for 100 replicates. Only bootstrap confidence levels more than 50% are shown [44].

and Far East virus, which are antigenically and genetically related but distinct from HTNV, were isolated from a patient with severe HFRS. An epizootiologic study indicated that the reservoir animals of the two viruses were *Apodemus peninsulae* and *A. agrarius*, respectively [47,48]. The deduced amino-acid sequences of the S and M segments of Amur virus showed 96.7% and 92.0–92.2% similarities with HTNV, respectively. For the Far East virus, the similarities were 99.1% and 97.9% in the S and M segments, respectively.

As noted above, HTNV isolated in China and Korea can be divided into nine distinct genetic subtypes [44]. Phylogenetic analysis of Amur virus and Far East virus together with analyses of other HTNVs indicated that both Amur virus and Far East virus belong to genetic subtypes that were previously identified amongst Chinese HTNVs. Two of the Chinese isolates with the same genetic subtype as Amur virus and the prototype HTNV strain 76118 showed more than a fourfold difference in cross-neutralisation tests to immune sera and to *A. peninsulae* sera obtained from an animal infected with Amur virus [49]. Therefore, the Amur virus and related viruses isolated in China may constitute a distinct serotype within the genus *Hantavirus* (Fig. 1). Recently, a new hantavirus, Khabarovsk virus, was isolated in reed voles

(*Microtus fortis*) in far-eastern Russia. Although the Khabarovsk virus is genetically most closely related to Puumala virus, its relationship to HFRS is still unclear [50].

In Korea, HTNV was first isolated in 1976 from the lung tissues of *A. agrarius*, captured along the Hantaan River [14]. With the eventual development of a hantavirus vaccine, the number of patients has decreased [51–53]; however, approximately 100–300 cases of HFRS, with an overall mortality of 4.5%, are reported annually [54]. Amongst all reported cases, approximately 70% and 20% are considered to be caused by HTNV and SEOV, respectively. In the remaining 10% of cases, the causative hantavirus has not been identified. Recently, a new hantavirus, designated Soochon virus, was isolated from *A. peninsulae* captured in the mountains of east-central Korea [54]. Phylogenetic analysis based on the almost entire sequences of the S and M segments indicated that Soochon virus is distinct from the HTNV virus of *A. agrarius*. Soochon virus is a member of the same clade as Amur virus and related viruses isolated in China, indicating that they shared a common ancestor virus. The results of cross-neutralisation tests with Soochon virus were similar to those obtained with the other viruses, suggesting that *A. peninsulae* HTNVs constitute a new hantavirus serotype.

An epidemiologic and epizootiologic study conducted in Russia, China and Korea indicated that *Apodemus*-borne HTNVs are the main causative agents of severe HFRS, although antigenic and genetic variability exist with respect to geographical distribution [48].

Although Japan borders those countries where HFRS is endemic, no large epidemics of the disease have been reported [55,56]. Both the urban rat (*R. norvegicus*) and laboratory rats were associated with cases of HFRS in the 1960s [57] and in the 1970s until 1984 [58], respectively, and during both episodes, the Seoul-type hantavirus was the causative agent. In the urban rat-mediated infections, 119 cases, two of which were fatal, were reported in an area with poor sanitary conditions. The endemic disappeared after conditions were improved. Infections associated with laboratory rats were reported by 21 institutions throughout Japan, and involved 126 cases, including one death. Since 1984, no cases of HFRS have been reported. However, SEOV-infected brown rats have been detected throughout Japan, mainly at port areas, and PUUV-infected grey red-backed voles (*C. rufocanus*) were identified in Hokkaido, northern Japan [59]. The limited number of seroepidemiological studies of human sera found that seropositive sera had been obtained from workers at a dumping-ground area (Tokyo), members of the Japanese self-defence force (Hokkaido) [55] and patients with hepatitis and renal failure of unknown etiology [60]. The virus was not isolated in any of these cases, but animals that were positive for the virus were suspected to be the sources of the infections. The PUUV genome amplified from the infected *C. rufocanus* in Hokkaido was most related to those obtained from the same species of voles captured in far-eastern Russia [61].

4.2. East Asian countries other than China, Korea and Japan

Seroepidemiological surveys confirmed hantavirus infections of humans and rodents in Taiwan [62,63], Hong Kong [64], Fiji [64], Malaysia [65], India [66],

Indonesia [67], Singapore [68], Sri Lanka [69], Thailand [70–72] and Vietnam [73]. In Myanmar and Australia [64], positive sera were obtained only from humans, whereas in Cambodia only positive rodents were detected [74]. Nonetheless, taken together, it is clear that hantavirus infections affect humans and rodents throughout Asia. Since the sizes of the epidemiologic studies varied depending on the country, the following section will be limited to a brief summary of the results from each country.

In Taiwan, the results of an extensive seroepizootiologic study of hantavirus infection amongst rodents were reported in 2000 [62]. From 1994 to 1995, 5461 rodents belonging to 16 species were collected throughout Taiwan and examined in an ELISA aimed at detecting anti-hantavirus antibodies. The most common host species belonged to the genus *Rattus*. A much higher antibody prevalence was found in urban regions (20%) than in rural areas (5%). Phylogenetic characterisation indicated SEOV infection. Unlike the situation in mainland China, no *A. agrarius* individuals positive for the virus were found (0/67). By May 2006, a total of seven cases of HFRS had been reported. However, the overall prevalence of hantavirus in Taiwan has yet to be examined.

In Thailand, *R. norvegicus* obtained from the port area of Bangkok was reported to be infected with hantavirus, probably SEOV, transported from abroad by ship [70]. In addition, various species of inland rodents are infected with hantavirus. Amongst them, the greater bandicoot rat (*Bandicota indica*) is a main reservoir of the virus, whilst several species of rice-field rats, such as *R. rattus*, *Rattus exulans* and *Rattus losea*, are also natural reservoirs albeit to a lesser extent. The Thailand virus (THAIV), one of the distinct species of virus within the genus *Hantavirus*, was isolated from *B. indica* captured in a village near the western province of Kanchanaburi in 1985 [70]. Therefore, THAIV or related viruses appear to be distributed throughout Thailand. Ten of the 30 sera obtained from residents of the village where virus-infected *Bandicota* was captured showed antibody to hantavirus, which demonstrated that Thailand virus is able to infect humans. However, the virulency of THAIV towards humans has not been determined. Hantavirus has been suspected as one of the pathogens in fevers of unknown origin (FUOs) in Thailand. In the period 1999–2000, 115 cases of FUO were reported in patients admitted to Bangkok Hospital who were examined for antibodies to hantavirus. Paired sera from one patient showed high antibody titres to HTNV by IgG ELISA, IgM ELISA and IFA test. Between 2002 and 2003, 260 paired sera from patients with FUO were collected in Surin Province. One of the sera showed a neutralising antibody titre to THAIV of 1:160, whilst the titres to HTNV and SEOV were less than 1:40. Furthermore, convalescent-phase serum did not contain hantavirus IgM antibody. Since the symptoms of the patient were comparable to those typical for HFRS, THAIV might be an additional causative agent of HFRS [72].

In Cambodia, a recent epidemiological study found that black rats, brown rats, and an unidentified *Rattus* species were infected with SEOV-like virus [74]. Furthermore, the viral genome amplified from seropositive black rats (*R. rattus*) was most related to that of THAIV [72]. Therefore, THAIV or a THAIV-like virus

circulates throughout Indochina and may also represent an additional causative agent of HFRS.

In Malaysia, positive sera were obtained both from renal patients and rats. Amongst the 119 sera from renal patients, 3 were positive for antibodies to HTNV, SEOV or Sin Nombre virus. In 14 of 87 rats, serum antibody titres were highest against SEOV [64].

In Indonesia, of the 94 patients from central Java with FUO, 5 were positive for hantavirus-specific IgM and IgG and another 5 were positive for HTNV- or SEOV-specific IgM, as determined by ELISA [75]. A more recent investigation of rodents, conducted on an island near Jakarta, showed that 43 of 185 *Rattus* sera were positive for SEOV (Dr. Ima, personal communication).

Positive sera, mostly to SEOV, from humans and from rodents were also reported in Vietnam [73]. Eight of 308 sera obtained from healthy people residing in the Haiphong port area and in HaNam Province, in northern Vietnam, were positive for SEOV. Four of 204 serum samples obtained from FOU patients living in the northern provinces of HaNam and ThanhHoa were likewise positive. Positive sera were also detected in *Rattus* individuals captured at the Haniphong port area and in HaNam and ThanhHoa provinces (Dr. Truong Uyen Ninh, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, personal communication).

In India, a high rate of positivity for hantavirus infection was reported. Of 152 sera obtained from patients with FOU, 23 were positive for IgM to either HTNV, SEOV or PUUV, as shown by ELISA, and 18 were positive by IFA. In sera from 87 healthy donors, two were shown to be positive by IFA [66].

More recently, sera positive for TPMV antibodies were found in 2 of 478 FOU patients in Thailand and in 2 of 14 *Suncus* sera obtained from animals captured on an island near Jakarta. Although these finding provide mainly serological evidence, the data indicate that TPMV is able to infect humans and can be maintained in the musk shrew as its natural host [76].

5. Conclusions

Epidemiological studies have shown that hantaviruses are widely distributed in Asia, both in humans and in rodents. Unlike the situation in Far East Asia, the number of hantavirus antibody-positive sera has so far been quite small, even amongst FOU patients. Therefore, the significance of hantavirus infection as the causative agents for FOU in East Asia remains unclear and further serological surveys amongst healthy people are needed. Nevertheless, these observations indicate that unidentified pathogens that cause FOU are prevalent in this region.

Epizootiologic studies, particularly amongst rodents, have confirmed the close relationship between hantavirus and rodents, which act as reservoirs for the virus. Since it thought that hantaviruses co-evolved with their rodent hosts, an understanding of the virus' ecology may provide unique and important information about other rodent-borne pathogens as causative agents of emerging infectious diseases.

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Analysis of the immune response of Hantaan virus nucleocapsid protein-specific CD8$^+$ T cells in mice

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Abstract

The major histocompatibility complex (MHC) class-I restricted epitope of Hantaan virus nucleocapsid protein (N) was identified using overlapping peptides and BALB/c mice. Using the MHC tetramer derived from the epitope, we found that the level of N-specific CD8$^+$ T cells increased to approximately 20% of all antigen-specific CD8$^+$ T cells in a mouse model of transient infection. However, N-specific CD8$^+$ T cells were undetectable in a mouse model of persistent infection, both in the persistently infected phase and in the convalescent phase. Levels of CD8$^+$ T cells producing interferon-γ were weak in both the acute and convalescent phases in the persistently infected model. These results indicate that hantavirus strongly suppresses the production of N-specific CD8$^+$ T cells throughout the course of infection in persistently infected mice. Moreover, N-specific CD8$^+$ T cells were not effective in recovering persistently infected mice, despite the existence of abundant N antigen *in vivo*.

Keywords: Hantavirus; MHC tetramer; Hantavirus-specific CD8$^+$ T cell; Persistent infection

Introduction

Hantaviruses comprise the genus *Hantavirus* in the family *Bunyaviridae*. Hantaviruses are spherical, enveloped viruses, with a diameter of 80–120 nm. The hantavirus genome consists of three segments of single-stranded, negative-sense RNA. The three segments have been designated the large (L), medium (M), and small (S) segments, based on their size. The L segment encodes an RNA-dependent RNA polymerase; the M segment encodes the surface glycoprotein precursor, which is cotranslationally cleaved into two glycoprotein spikes (Gn and Gc); and the S segment encodes the nucleocapsid protein (N) (Lednicky, 2003).

To date, 22 virus species have been classified in the genus *Hantavirus* based on antigenic, genetic, and ecological characteristics. Rodents are the natural reservoirs of all but one species, the Thottapalayan virus, whose reservoir animal is the insectivore *Suncus murinus* (Carey et al., 1971). Because they are species-specific, it is generally believed that hantaviruses have coevolved with their hosts, and nearly identical phylogenetic trees can be constructed from host mitochondrial DNA sequences and viral RNA sequences (Meyer and Schmaljohn, 2000). Hantaviruses cause two serious and often fatal human diseases, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Transmission of hantaviruses to humans does not require direct human-rodent contact. Instead, humans typically become infected by inhaling virus-contaminated aerosols of rodent excreta, such as feces, saliva, or urine (Lee and van der Groen, 1989).

Because hantaviruses have no, or an incomplete, cytopathic effect on cultured cells (Ogino et al., 2004) and are generally non-lytic (Pensiero et al., 1992; Yanagihara and Silverman, 1990; Zaki et al., 1995), the pathogenesis of HFRS and HPS may be related to immune-mediated effector responses of the host itself. Indeed, several studies have suggested that hantavirus infection can induce a vigorous cellular immune response in humans (Chen and Yang, 1990; Huang et al., 1994; Kilpatrick et al., 2004; Mustonen et al., 1994; Nolte et al., 1995;

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