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GEOGRAPHICAL DISTRIBUTION OF HANTAVIRUSES IN THAILAND AND POTENTIAL HUMAN HEALTH SIGNIFICANCE OF *THAILAND VIRUS*

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Abstract. Phylogenetic investigations, sequence comparisons, and antigenic cross-reactivity studies confirmed the classification of *Thailand virus* (THAIV) as a distinct hantavirus species. The examination of sera from 402 rodents trapped in 19 provinces of Thailand revealed that five greater bandicoot rats (*Bandicota indica*) and one lesser bandicoot rat (*B. savilei*) from four provinces were focus reduction neutralization test (FRNT) antibody-positive for THAIV. One of 260 patients from Surin province in Thailand (initially suspected of having contracted leptospirosis, but found to be negative) showed symptoms compatible with hemorrhagic fever with renal syndrome (HFRS). The serum of this patient showed high titers of hantavirus-reactive IgM and IgG. FRNT investigations confirmed virus-neutralizing antibodies against THAIV. These observations suggest that THAIV or THAI-like viruses occur throughout Indochina and may represent an additional causative agent of HFRS.

INTRODUCTION

Hemorrhagic fever with renal syndrome (HFRS) is a rodent-borne viral zoonosis caused by certain members of the viruses in the genus *Hantavirus* of the family *Bunyaviridae*.[1] The hantaviral species that have been causally associated with HFRS are *Hantaan virus* (HTNV), *Seoul virus* (SEOV), and *Dobrava/Belgrade virus* (DOBV) that are carried by the members of the rodents in the subfamily *Murinae* (Old World rats and mice), while the *Puumala virus* (PUUV) is carried by the members of the rodents in the subfamily *Arvicolinae* (voles and lemmings) of the family *Muridae*. Other hantaviral species that are not known as the causative agents of HFRS include *Tula virus* (TULV) and *Topografov virus* (TOPV) in Europe, *Khabarovsk virus* (KHAV) in far east Russia, and *Prospect Hill virus* (PHV) in the United States that are carried by rodents in the subfamily *Arvicolinae*. The *Thailand virus* (THAIV) is the only hantavirus species carried by the rodent in the subfamily *Murinae* in Thailand. *Thottapalayam virus* (TPMV) is the only hantavirus isolated from mammals in the Insectivore in India.[2]

The species of hantaviruses isolated from the rodents in the same subfamily; HTNV, SEOV, DOBV, and THAIV from rodents of subfamily *Murinae* and PUUV, TULV, TOPV, KHAV, and PHV from rodents of subfamily *Arvicolinae*, showed strong antigenic cross reactivity defined by antibody binding assays such as IFA and ELISA. Neutralization test is required to serologically distinguish among hantavirus species originated from rodents classified to the same subfamily.[3]

The THAIV strain Thai749 was originally isolated by Elwell et al. (1985) from a greater bandicoot rats (*Bandicota indica*) trapped in the vicinity of a small farm village in the western province of Kanchanaburi,[4] Thailand. Subsequent phylogenetic studies based on the nucleotide sequence of M segment of THAIV revealed that the THAIV is placed at the position most closely related to SEOV and grouped with other viruses from rodents classified to *Murinae*.[5] Thai749 strain is antigenically distinct from other hantavirus species.[3] However, only part of the nucleotide sequence information in the S segment of the THAIV is available so far.[5] For further understanding of THAIV of the relationship among other hantaviruses, nucleotide sequence information of entire S segment as well as further antigenic characterization is required.

It has been well characterized that a single rodent species or phylogenetically closely related rodent species are the principal host of a single hantavirus species.[6] The rodent fauna of Thailand includes 35 murine species in 7 genera and 1 arvicoline species, *Eothenomys melanogaster*.[7] A previous seroepizootiologic study of hantavirus infection conducted at central, northeastern, and near Bangkok areas revealed that greater bandicoot rat as a main reservoir and several species of rice field rats such as *Rattus rattus*, *exulans*, and *losea* are also natural reservoirs to a lesser extent in Thailand.[4] To extend our knowledge of the geographical distribution and natural host association of the hantaviruses in Thailand, we have continued further seroepizootiologic study, particularly by including the THAIV as antigen for serological screening.

Although the hantavirus infection spread in various species of rodents and wider areas in Thailand, epidemiologic information regarding to the human infection with hantavirus is quite limited. Suputthamongkol et al.[8] reported the first clinical case report of hantavirus infection in Thailand. However, the causative hantavirus species was not further characterized in the report. Since the clinical symptoms of leptospilosis and other febrile illness are similar to HFRS, undiagnosed HFRS cases would be existing among the patient with febrile illnesses of unknown etiology in Thailand.

In this study, we have examined antigenic and genetic prop-

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erties of THAIV to provide new insights into the relatedness of THAIV to other hantavirus species and confirm the classification of THAIV as a distinct hantavirus species. In addition, serological surveillance of hantavirus infection among rodents indicated the prevalence of THAIV infection mainly among giant bandicoot rats and *Rattus* species in Thailand. Finally, we present the first case of an infection with THAIV or a hantavirus antigenically more closely related to THAIV than to HTNV, SEOV, or DOBV in a human who showed symptoms consistent with HFRS.

MATERIALS AND METHODS

Viral strains and cells. *Hantaan virus* (HTNV) strain 76-118 and SEOV strain SR-11 were used as representative strains of the HTNV and SEOV species, respectively. The THAIV strain Thai749 was a gift from Dr. P.W. Lee of the WHO Collaborating Center for Virus Research for Hantaviruses in Korea. All of the viruses were propagated in Vero cells (clone E6; ATCC C1008) prior to molecular and antigenic characterizations or use in FRNT. The DOBV strains Slovenia[9] and Saaremaa-DOE,[10] SEOV strain Gou3,[11] and HTNV strain *Da Bie Shan virus* (DBSV)-NC167 isolated from *Niviventer confucianus* captured in a mountainous region near Dabishan, Anhui Province, China,[11] were used for antigenic comparisons.

Monoclonal antibodies (MAbs). Clones that produce MAbs directed against the HTNV envelope glycoproteins and N protein were prepared as previously described.[12,13]

Nucleotide sequence determination and phylogenetic analysis. Hantaviruses possess a negative-sense RNA genome that consists of 3 segments, which are designated as large (L), medium (M), and small (S). The L segment encodes the RNA-dependent RNA polymerase. The M segment encodes a glycoprotein precursor that is co-translationally cleaved into the G1 and G2 envelope glycoproteins, and the S segment encodes the nucleocapsid (N) protein.[1] The nucleotide sequence of the M segment has been published, but not in L and S segment. Total RNA was isolated from THAIV-infected Vero E6 cells, and hantavirus-specific cDNA was synthesized, as previously described.[11] To amplify the partial M genome segment that corresponds to nucleotides (nt) 2000–2300, the primer pair THLM1910F, (5′-AAAAGCAGATGTTACAT-3′) and THLM2364R (5′-TTTTCAAGTGACACTT-3′) was used. The entire S genome segment was amplified as 2 overlapping PCR products nt 1–1220 and nt 1025–1885 by using the two primer pairs CS1 (5′-TAGTAGTAGACTCCCT-AAAGAGCTAC-3′) and GS6 (5′-AGCTCIGGATCCAT-ITCAT-3′), as well as GS4 (5′-GAIIGITGTCCACCAA-CATG-3′) and CS8 (5′-TAGTAGTAGGCTCCCTAAA-AAGACAA-3′).[11,14] The PCR product of the expected size derived from the partial M segment was purified using a PCR purification kit (Qiagen, Hilden, Germany) and sequenced with the same primers that were used for the PCR amplification. The PCR products derived from the S genome segment were cloned into an *E. coli* vector using the Original TA Cloning Kit (Invitrogen, Carlsbad, CA). Two clones of each amplification product were sequenced with M13-forward and -reverse primers. The sequencing reaction was performed with dye terminator reactions using a BigDye Terminator Cycle Sequencing Kit version 3.1 (Perkin Elmer, Applied Biosystems Division, Foster City, CA). The samples were sequenced on model 3100 DNA Sequencing System (Perkin Elmer, Applied Biosystems Division). The sequences obtained from 2 independent clones for each PCR amplification product were found to be identical. Although the almost complete S segment nucleotide sequence (except the extreme 5′- and 3′-termini covered by the amplification primers) was obtained, only the entire N protein coding sequences that allowed unambiguous alignment were used for the phylogenetic analysis.

The sequences were aligned using CLUSTALW[15] with the default parameters. The reliability of the alignment was checked using DotPlot analysis implemented in the BioEdit (Carlsbad, CA) software package (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The alignment was tested for phylogenetic information by likelihood mapping analysis.[16] In the subsequent phylogenetic analyses, the maximum likelihood (ML) and neighbor-joining (NJ) phylogenetic trees were calculated. To reconstruct the ML phylogenetic trees, a quartet puzzling algorithm implemented in the TREE-PUZZLE 5.2 package[16,17] was applied. The Tamura-Nei and Hasegawa-Kishino-Yano evolutionary models were used for the tree reconstructions. Missing parameters were reconstructed from the datasets. NJ trees with the Tamura-Nei evolutionary model were constructed using the PAUP* 4.0 Beta 10 software package (Sunderland, MA).[18] In addition, bootstrap analysis with 1,000 replicates was performed to evaluate the statistical support of the topology for the derived tree. The resulting evolutionary trees were then visualized using TreeView (Glasgow, UK) v.1.6.6 (http://taxonomy.zoology.gla.ac.uk/rod/ treeview.html). The accession numbers of the sequences used in the phylogenetic analysis are listed in the legend to Figure 1. The sequence of the S segment of the THAIV strain Thai749 has been deposited into the GenBank nucleotide sequence database with accession number AB186420.

Indirect immunofluorescent antibody (IFA) assay. Since the HANTADIA assay showed weak agglutination pattern in some of the sera, we also used IFA test for screening test. The indirect immunofluorescent antibody (IFA) assay was performed as described previously.[19] Briefly, acetone-fixed smears of Vero E6 cells infected with hantaviruses were used as antigens. For the antigenic comparison of THAIV with other hantaviruses by using the MAbs (Table 1), HTNV strains 76-118, AMRV-H5, and DABV-NC167, SEOV strains SR-11 and Gou3, DOBV strains Slovenia, and Saaremaa-DOE, and THAIV strain Thai749 were used. Fluorescent isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin G (H and L chains) (Zymed Laboratories Inc., South San Francisco, CA) was used as the secondary antibody. The serum specimens that showed characteristic fluorescence in the infected Vero cells but negative with uninfected Vero cells were regarded as positive.

Focus reduction neutralization test (FRNT). The endpoint titers of neutralizing antibodies against HTNV strain76-118, SEOV strain SR-11, and THAIV strain Thai749, were determined by FRNT, as described earlier.[20] For this purpose, we selected seropositive sera from human and rodent sera. Human sera and rodent sera derived from trapping point #1 to #13 (Figure 2, Table 2) positive by ELISA were selected. And rodent sera derived from trapping point #14 to #22 positive by both HANTADIA and IFA were selected for further investigation. However a *Rattus rattus* serum from Phetchaburi was not used for FRNT assay because its amount was not suffi-

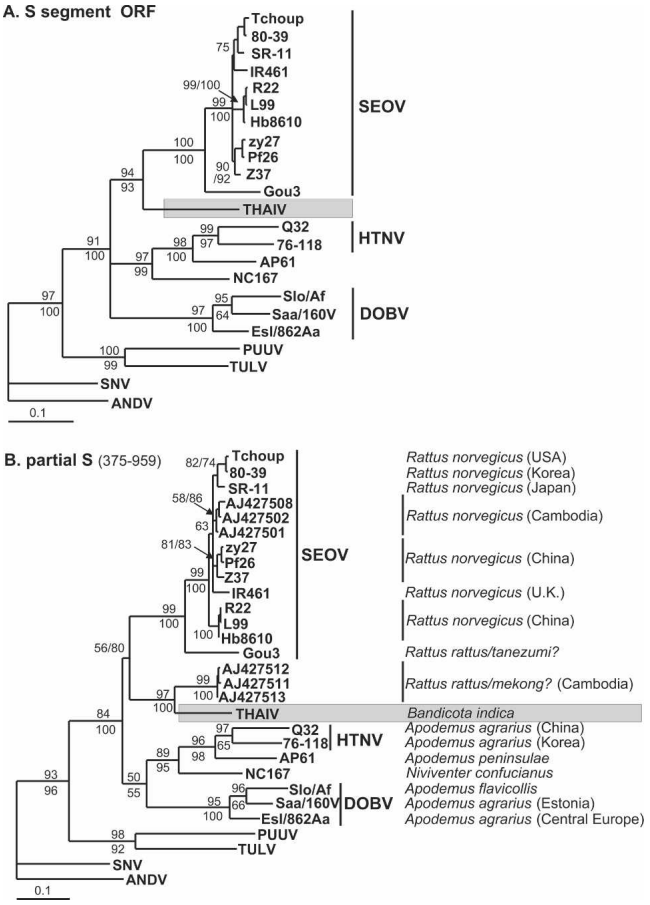

FIGURE 1. Maximum likelihood (ML) phylogenetic trees of THAIV and other Murinae-associated hantaviruses based on: (**A**) complete N protein coding nt sequences (S segment ORF); and (**B**) partial S segment nucleotide sequences of nt 375–959 (585 nts). The ML trees (Tamura-Nei evolutionary model) were calculated using TREE-PUZZLE package. The values above the branches represent PUZZLE support values. The values below the branches are the bootstrap values of the corresponding NJ tree (Tamura-Nei evolutionary model) calculated with the PAUP* software from 1,000 bootstrap replicates. THAIV is marked by a gray box. The S segment sequences that were analyzed included THAIV (AB186420), SEOV/SR11 (M34881), SEOV/Tchoupitoulas (AF329389), SEOV/80-39 (AY273791), SEOV/IR461 (AF329388), SEOV/R22 (AF488707), SEOV/L99 (AF488708), SEOV/Hb8610 (AF288643), SEOV/zy27 (AF406965), SEOV/Pf26 (AY006465), SEOV/Z37 (AF187082), Gou3 (AB027522), HTNV/76-118 (M14626), HTNV/Q32 (AB027097), Amur virus AMRV/AP61 (AB071183), DBSV/167 (AB027523), DOBV/Slo/Af (L41916), DOBV/Esl/862Aa (AJ269550), Saaremaa/160V (AJ009773), PUUV/CG1820 (M32750), Tula virus strain Moravia/5302v (Z69991), Sin Nombre virus strain NM H10 (L25784), and Andes virus strain Chile-9717869 (AF291702). In the lower tree (B), the partial sequences of Cambodian hantavirus strains detected in *Rattus rattus* (AJ427511-AJ427513) and in *R. norvegicus* (AJ427501, AJ427502, AJ427508) were added to the dataset. The natural rodent species (subfamily Murinae) of the corresponding hantavirus strains are listed.

cient. Briefly, 100 μL of serial 2-fold dilutions of serum were mixed with an equal volume of virus suspension containing 200 focus-forming units (FFU) of virus at 37°C for 1 hr. Fifty microliters of the mixture was then inoculated onto Vero E6 cell monolayers in 96-well tissue culture plates (IWAKI 3860-096, Asahi Technoglass Co., Tokyo, Japan). After adsorption for 1 hour at 37°C, the wells were overlaid with medium that contained 1.5% carboxymethyl cellulose. After being incubated for 7 days in a CO_2 incubator, the monolayers were fixed with acetone-methanol (1:1) and dried. The foci of the virus-infected cells were detected by staining with a polyclonal antiserum from a rabbit that was immunized with the truncated N protein (amino acids 1–244) of HTNV, followed by the addition of horseradish peroxidase-labeled goat antibodies and substrate. The FRNT titer was expressed as the reciprocal of the highest serum dilution that resulted in a > 80% reduction in the number of infected cell foci.

Rodent sera and antibody detection. In total, serum samples from 402 different rodents were collected from 22 locations in 19 provinces of Thailand from 1995–1998 (Figure 2, Table 2). Distinction of rodent species examined in the present study followed morphologic criteria including dental morphology and coloration of phage outlined by Corbet and Hill,[21] Musser and Brothers,[22] and Marshall.[7] The blood samples were taken after the animals were anesthetized with CO_2 and taxonomically identified; the weight, sex, and locality of collection were recorded. Then, the animals were euthanized with CO_2. Most of the captured rodents were brought to the Institute. The cadavers were incinerated at the Institute. Serum samples derived from trapping sites #1 to #13 were tested in an indirect IgG ELISA using yeast-expressed His-tagged SEOV, strain 80-39, recombinant N protein.[23] Briefly, polystyrene microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) were coated overnight at 4°C with 2 μg/ml recombinant N protein from SEOV diluted in 0.05 M carbonate buffer (pH 9.8). Blocking of the plates was accomplished by the addition of 3% bovine serum albumin (BSA)/0.05% Tween-20 in PBS followed by the addition of rodent serum samples diluted 1/200 with 1% BSA/0.05% Tween-20 and horseradish peroxidase (HRP)-conjugated goat anti-rat IgG (Sigma Chemical Co., St. Louis, MO). To detect immunoreactivity, the *o*-phenylenediamine (OPD) substrate was added, and the reaction was stopped by the addition of 100 μL of 1 M H_2SO_4. Finally, the optical density (OD) was measured at 492 nm (reference, 620 nm). The final OD value for each serum sample was calculated as the difference of the OD values for antigen-containing and antigen-free wells. These final OD values for serum dilutions of 1/200 were regarded as positive if they exceeded the cutoff value of 0.270 determined by investigation of non-infected and experimentally SEOV-infected rats.[23,24] The serum samples derived from trapping sites #14 to #22 were screened using a commercial agglutination test based on inactivated HTNV antigen (HANTADIA®; Korea Green Cross Corp., Seoul, Korea) and an indirect immunofluorescent antibody (IFA) test. In HANTADIA screening, sera were screened by the manufacturer's instructions at 1:40 dilution. Serum specimen that showed clear agglutination was regarded as positive. In IFA test, the sera were examined at 1:40 dilution with HTNV strain 76-118-infected Vero E6 cell smears as antigen. As negative control, each serum sample was tested with uninfected Vero E6 cells. The serum specimen that showed characteristic fluorescence in the infected Vero E6 cells but negative with uninfected Vero E6 cells was regarded as positive. Antibody-positive sera from both screenings were confirmed by Western blotting using recombinant hantavirus N proteins of HTNV strain 76118 as previously described.[23,25,26] As positive controls, 3 serum samples from Wistar rats that were experimentally in-

TABLE 1
Antigenic profiling with N-, G1-, and G2-specific MAbs of THAIV and other murinae-associated hantaviruses*

			HTNV			SEOV		DOBV		THAIV
Proteins	Epitope	MAbs	HTNV 76118	AMRV H5	DBSV NC167	SEOV SR-11	SEOV Gou3	DOBV Slovenia	DOBV Saaremaa	THAIV Thai749
N	Cross-reactive	ECO2	+++	+++	+++	+++	+++	+++	+++	+++
	Genus-common	E5G6	+++	+++	+++	+++	+++	++	++	+++
	HTNV-specific	BDO1	+++	+++	+++	–	–	–	–	–
	SEOV-specific	DCO3	–	–	–	+++	+++	–	–	–
G1/G2	G1a	6D4	+++	+++	+++	–	–	+++	+++	+++
	G1b	3D5	+++	+++	+++	–	–	–	–	–
	G2a	HCO2	+++	+++	+++	+++	+++	–	–	+++
	G2b	EBO6	+++	+++	+++	+++	+++	±	±	–
	G2c	11E10	+++	+++	+++	–	–	+++	–	–
	G2d	3D7	+++	++	+++	+++	+++	+++	+++	+++
	G2e	20D3	+++	+++	–	+	+	++	++	+++
	G2f1	1G8	+++	++	+++	+++	+++	+++	+++	+++
	G2f2	7G6	+++	++	+++	+++	+++	++	+++	+++

Binding profiles of clones data not shown in this table were basically same results as representative clones and previous reports.[13] All the used clones were listed as below. Cross-reactive clones for N protein: ECO2, FDO3, KAO6, ECO1, GBO4, C16D11, and F23A1; Genus-common epitope binding clone: E5G6; HTNV-specific clones for N protein: BDO1, C24B4, and G5; SEOV-specific clone for N protein: DCO3.

Clones for glycoprotein epitope G1a: 6D4, 8B6, and 10F11; G1b: 3D5, 2D5 and 16D2; G2a: HCO2 and 16E6, G2b: EBO6; G2c: 11E10; G2d: 3D7; G2e: 20D3, 17G6 and 5B7; G2f1: 1G8, 8E10, 1C6, 23G10-2, and 3B6; G2f2: 7G6, 23G10-1, and 18F5. Designations: –, $< 10^2$; +, 10^2; ++, 10^3; +++, 10^4; ±, weak positive reaction at dilution of 1:100.

fected with SEOV strain SR-11 were used.[26] As negative controls, sera from 5 wild-trapped, non-infected rats from Japan were used.[24]

Human sera and methods for antibody detection. Screening for anti-hantavirus IgG and serotyping were performed by ELISA tests, as previously described,[20] using recombinant entire and truncated N protein antigens expressed by recombinant baculovirus. Briefly, serum specimens were screened with the dilution of 1:200. As a negative control antigen, bornavirus P24 antigen expressed by baculovirus was used. Recombinant N proteins of HTNV (strain 76-118), and PUUV (strain Sotkamo) and truncated N proteins of HTNV (strain 76-118), SEOV (strain SR-11), and DOBV (strain Saaremaa-DOE) were expressed from baculovirus vectors. The screening for virus-reactive IgM was performed with the μ-capture ELISA, as described previously.[27] Positive results were confirmed by IFA testing using SEOV-infected Vero E6 cell antigen and by Western blotting using recombinant HTNV antigen. Three types of positive control sera from HFRS patients who had been previously diagnosed by FRNT as being infected with HTNV, SEOV, and PUUV, and negative human control sera (NHS), which were confirmed to contain no antibodies against hantaviruses, were used.[20,28]

RESULTS

Genetic characterization of Thailand virus. The nucleotide sequences of the entire M genome segment and partial S genome segment of the THAIV strain Thai749 have been published (GenBank accession numbers L08756 and U00471[5]). Partial M segment sequence of the THAIV obtained in this study was completely identical with the published sequence. To characterize genetically the THAIV strain Thai749 in more detail, we cloned and sequenced entire S genome segment except primer binding region (GenBank accession number AB186420). The sequences of 2 independent clones for each of the PCR amplification products were found to be identical. The deduced amino acid sequence identity on comparison of the N protein of THAIV to those of SEOV, HTNV, and DOBV are calculated as 86.5%, 83.7%, and 81.6%, respectively. The previously determined values for sequences of THAIV glycoprotein precursors[5] showed amino acid sequence identity to those of SEOV, HTNV, and DOBV as 73.3%, 71.3%, and 71.2%, respectively. Thus, the N protein amino acid sequence information also meet one of the criteria set forth in the Eighth Report of the International Committee on Taxonomy of Viruses for species demarcation within the genus *Hantavirus* (more than 7% difference).[1,29] The phylogenetic analysis (Figure 1A) based on the nucleotide sequence of the N protein-encoding open reading frame (ORF) of the S genome segment revealed that THAIV was clearly placed in a distinct lineage within a single cluster with SEOV, HTNV, and DOBV, which are associated with the rodent reservoirs classified into the murid subfamily *Murinae*. Since *B. indica* is classified to the *Murinae* subfamily, the observed lineage of THAIV is in accordance with the host-virus co-evolution theory for hantaviruses.[30,31] As shown in Figure 1B, phylogenetic analysis based on a partial nucleotide sequence (nt 375–959) in the central region of the S segment, which contains the highly variable region, reveals that THAIV is most closely related to Cambodian virus strains isolated from *R. rattus*. [32]

Antigenic characterization of Thailand virus using monoclonal antibodies. To clarify the antigenic characteristics of THAIV, 34 MAbs, including 12 against the N protein and 22 against the G1 or G2 envelope proteins, were used to compare the antigenic profiles of the THAIV prototype strain Thai 749 and other hantaviruses using IFA (Table 1). The antigenic profiles of HTNV strains 76118 and Amur virus (AMRV)-H5 were taken from a previous report.[33] Among the MAbs directed against the N protein, cross-reactive clones to HTNV, SEOV, and DOBV-types were also reactive against THAIV. On the other hand, the HTNV-type specific and SEOV-type specific clones for N protein were not reactive against THAIV. Similarly, HTNV-type specific anti-G1 MAb (3D5) did not react to THAIV. However, the rest of clones showed variable cross reactivities among the 4 types of viruses. Therefore, in spite of the close antigenic relationships between hantaviruses that are associated with *Murinae* reservoir hosts, the antigenicity of THAIV was distinct from the

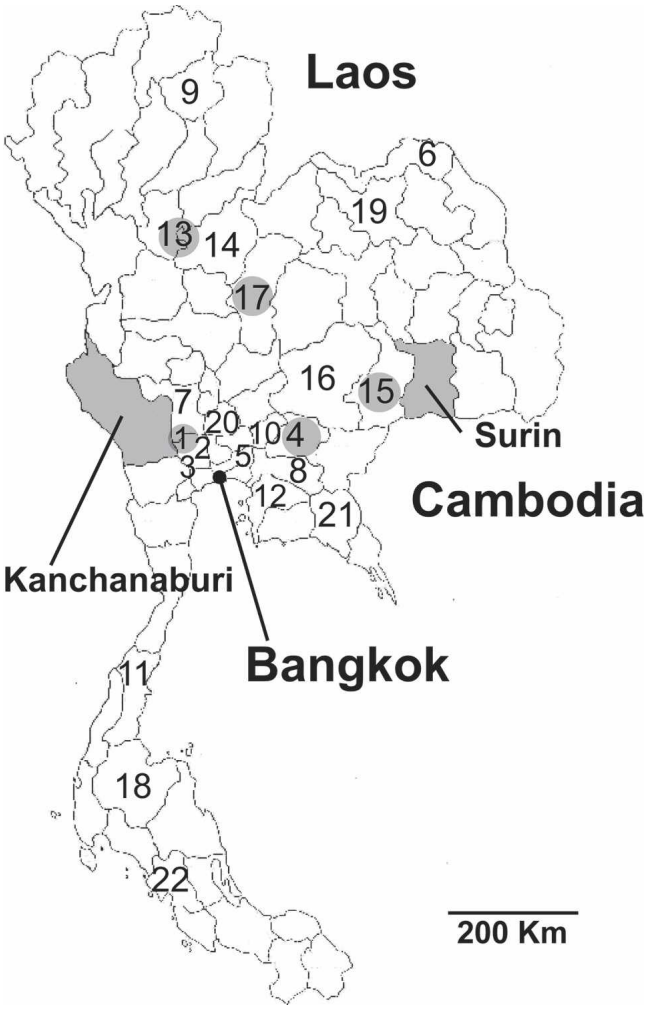

FIGURE 2. Map of Thailand showing the localization of rodent trapping sites. The numbers of the trapping sites correspond to those given in Table 1. The locations of the trapping sites where anti-hantavirus–positive rodents were captured are shown as gray circles. The geographical origin of patient #277 in Surin Province is shown as a gray area. The locality of collection of the *Bandicota indica* used for isolation of THAIV strain Thai749, in the western province Kanchanaburi is given as gray area.

other Murinae-associated hantaviruses. These results were corresponding to the previous report from Dr. Chu et al. [3]

Serological survey of rodent sera. In total, serum samples from 402 rodents captured at 22 different sites (Figure 2) were examined for IgG reactive against hantavirus antigens. Serological screening was carried out by ELISA or with an agglutination kit (HANTADIA). As shown in Table 2, 7 of 402 (1.7%) serum samples were antibody-positive. Of the 7 seropositive specimens, 5 were derived from *B. indica*, one from *B. savilei*, and one from *Rattus rattus*. The 5 provinces in which the seropositive rodents were located (i.e., Nakhon Pathom, Prachin Buri, Phitsanulok, Buri Ram, and Phetchabun) are distributed close to Bangkok in the eastern and northern parts of Thailand (Table 2, Figure 2).

To characterize the apparent homologous virus, 5 positive *Bandicota* sera were selected, and FRNT investigations were performed (Table 3). All of the rodent sera showed the highest FRNT titers to THAIV, which indicates that THAIV or THAI-like viruses exist among rodents in Thailand. Two other positive sera, one from *B. indica* and one from *R. rattus*, were not available for the FRNT due to an insufficient amount of serum.

TABLE 2
Trapping sites, collected rodent species and seropositivity for hantavirus

Site no.	Trapping site	Rodent species	No. of seropositives/ No. of animals tested
1	Nakhon Pathom/Bang Len	*Rattus rattus*	0/9
		Bandicota indica	**1/83**
2	Nakhon Pathom/Nakhon ChaiSi	*R. rattus*	0/1
3	Nakhon Pathom/Sam Phran	*R. rattus*	0/1
4	Prachin Buri	*R. losea*	0/7
		B. indica	**2/18**
5	Bangkok Metropolis	*R. norvegicus*	0/16
		R. rattus	0/9
6	Nong Khai	*R. rattus*	0/1
7	Suphan Buri	*R. argentiventer*	0/11
8	Chanchoengsao/Bang Nam Prieo	*R. norvegicus*	0/34
		R. rattus	0/6
		B. indica	0/34
9	Phayao	*R. rattus*	0/1
10	Nakhon Nayok	*R. rattus*	0/1
11	Chumphon/Bang Son (Pathiu)	*R. tiomanicus*	0/97
12	Chon Buri	*B. indica*	0/2
		B. savilei	0/1
		R. rattus	0/2
		R. exulans	0/1
13	Phitsanulok/Phrom Piram	*R. argentiventer*	0/19
		R. losea	0/4
		B. savilei	**1/3**
14	Phitsanulok	*B. indica*	0/7
		R. rattus	0/6
15	Buri Ram	***B. indica***	**2/3**
		R. rattus	0/2
16	Nakhon Ratchasima	*B. indica*	0/2
		R. rattus	0/1
17	Phetchabun	*B. indica*	0/3
		R. rattus	**1/1**
18	Surat Thani	*R. rattus*	0/2
19	Udon Thani	*R. rattus*	0/1
		R. exulans	0/2
20	Ayutthaya	*B. indica*	0/1
		R. rattus	0/1
		R. exulans	0/2
21	Chanthaburi	*R. rattus*	0/1
		R. norvegicus	0/2
22	Trang	*R. rattus*	0/2
		R. exulans	0/2
	All	***B. indica***	5/152 (3.3%)
		B. savilei	1/5 (20.0%)
		R. rattus	1/48 (2.1%)
		R. exulans	0/7
		R. norvegicus	0/52
		R. losea	0/11
		R. argentiventer	0/30
		R. tiomanicus	0/97
	Total		7/402 (1.7%)

The groups in which the positive rodents were detected are shown by the bold-faced type.

Serological survey of human sera. A total of 260 paired sera were obtained from 260 patients who were clinically diagnosed with leptospirosis but were serologically negative for *Leptospira* antigens. Two paired sera (#53 and #54, #277 and #277/2004) showed positive reactions against the HTNV antigen but negative or very low reactivity against the PUUV antigen (Figure 3A). The ELISA OD values of anti-hantavirus IgG in serum #53 and #54 were 0.309 and 0.398,

TABLE 3

Analysis of human and rodent serum samples in focus reduction neutralization test (FRNT) using *Hantaan* virus (HTNV), Seoul virus (SEOV), and *Thailand* virus (THAIV)

Serum specimen/antiserum	Reciprocal end-point titer† against		
	HTNV	SEOV	THAIV
A172 *(Bandicota indica)*	< 40	< 40	**80**
Bi65 *(B. indica)*	< 40	< 40	≥ **1280**
Bi74 *(B. indica)*	< 40	< 40	≥ **1280**
Bi324 *(B. indica)*	< 40	< 40	**80**
Bs355 *(B. savilei)*	< 40	< 40	**160**
Anti-HTNV/mice	≥ **1280**	< 40	< 40
Anti-SEOV/rat	< 40	≥ **1280**	80
Negative sample of *B. indica*	< 40	< 40	< 40
#277	40	< 40	**160**
Anti-HTNV	**640**	< 40	< 40
Anti-SEOV	80	**640**	160
NHS‡	< 40	< 40	< 40

† The highest neutralizing antibody titer for each serum is given in bold.
‡ Serum sample from a healthy human individual.

respectively. The virus-specific IgM was not detected (Figure 3A, 3B). Therefore, this patient may have been infected with a hantavirus many years ago and was suffering from an illness that was unrelated to recent hantavirus infection. Serum #277 contained high concentrations of HTNV-reactive IgM and IgG (Figure 3A, 3B). The #277/2004 serum, which was collected 12 months after the onset of disease, showed high IgG concentration but quite lower IgM concentration. The presence of anti-hantavirus antibodies in serum #277 and #277/2004 was also confirmed by IFA testing using SEOV-infected Vero E6 cells and by Western blotting using recombinant HTNV N protein antigen (data not shown). The detection of HTNV-reactive IgM in patient serum #277 in acute phase but not in convalescent phase may represent an indication of an hantavirus infection.

The serotyping of serum #277 by ELISA revealed reactivities to the truncated N proteins of HTNV, SEOV, and DOBV. However, unlike the positive control sera, serum #277 was equally reactive against the 3 test antigens (Figure 3C). This may indicate that the patient with serum #277 was probably infected with a hantavirus other than HTNV, SEOV, and DOBV. To further characterize the antibody response of serum #277, neutralizing capacity against HTNV, SEOV, and THAIV was tested using FRNT (Table 3). The results indicated that the patient with serum #277 was infected with either THAIV or a THAI-like virus, since the neutralizing antibody titer against THAIV was at least 4-fold higher than that against HTNV or SEOV.

The clinical profile of the patient with serum #277 was consistent with HFRS. The male patient was a 26-year-old farmer from Surin province in northeastern Thailand who was admitted to a mobile "fever unit" with a 40°C fever that had developed over the previous days. The physical examination on admission showed a well orientated patient who suffered from headache, abdominal pain, and conjunctival suffusion. Urine analysis displayed a proteinuria, glucosuria, erythrocyturia, and leukocyturia. The serum level of the alanine aminotransferase was 110 IU/l, the aspartate aminotransferase level was 240 IU/l, and the alkaline phosphatase level was 480 IU/l. The patient showed neither hemorrhages nor oliguria. The serological tests performed for leptospirosis, dengue fever, influenza, and scrub typhus were negative.

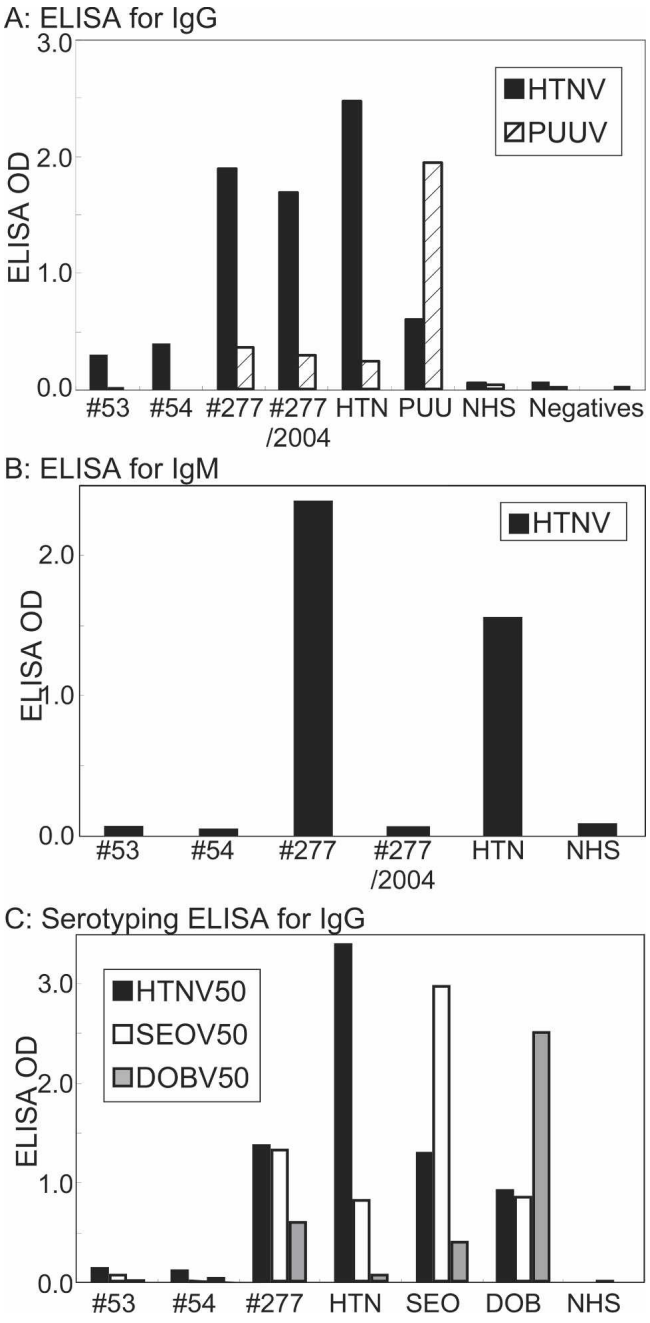

FIGURE 3. Serological screening of patient sera obtained in Thailand. (**A**) Detection of anti-hantavirus IgG in patient sera #53, #54, #277, and #277/2004 by ELISA using the recombinant N protein antigens of HTNV (closed bar) and PUUV (hatched bar). HTN and PUU are positive control sera from patients who were previously confirmed to be infected with HTNV in China and with PUUV in Sweden, respectively. NHS represents a human serum sample that was confirmed as negative for hantavirus-specific antibodies. Typical negative reactions in the initial screening assay are shown as negative. (**B**) Detection of anti-hantavirus IgM by μ-capture ELISA using the recombinant N protein antigen of HTNV. HTN is a positive control serum sample of the acute phase from a patient previously confirmed as being infected with HTNV. (**C**) Serotyping ELISA for human sera #53, #54, and #277 using truncated N antigens (amino acids 50-429) of HTNV (HTNV50), SEOV (SEOV50), and DOBV (DOBV50). Human sera HTN, SEO, and DOB were used as positive controls in the assay and have been characterized previously.

All the methods used showed basically the same tree topology. Therefore, only the ML trees with the Tamura-Nei evolutionary model are shown. We obtained a total of 260 paired sera (acute phase and convalescent phase) from different patients who had a fever of unknown etiology and were found to be seronegative for leptospirosis, dengue fever, influenza, and scrub typhus. The sera were collected in Surin Province of Thailand (Figure 2) in 2002 (454 sera), 2003 (65 sera), and 2004 (1 serum).

DISCUSSION

To further characterize the genetic and antigenic relatedness of THAIV to other Asian hantavirus species, first we cloned and sequenced the almost entire S genome segment of THAIV strain Thai749. Our sequence and phylogenetic analysis based on the nucleotide sequence of the N-protein-encoding ORF on the S segment revealed the same conclusions as previously drawn from complete M segment analyses; THAIV is most closely related to the SEOV species but different enough to appear as a distinct branch on the phylogenetic tree.[5,11] The different aa sequence similarities are reflected also in the reactivity of N-, G1-, and G2-specific MAbs with the corresponding proteins of THAIV and other hantaviruses. In general, our IFA reactivities of all anti-G1 and anti-G2 MAbs with Thai749 are in line with data of ELISA investigations published previously. In contrast, the reactivity of these MAbs in hemagglutination inhibition assay and especially plaque reduction neutralization test (PRNT) differed markedly to our IFA data, most likely due to the differences of the test formats.[3] The definition of THAIV as a distinct species was based on its association to a unique rodent species (i.e., *B. indica*).[4] Recently, THAIV genome was amplified by RT-PCR from *B. indica* captured in central Thailand (personal communication from Alexander Plyushin). In addition, the 2-way cross-neutralization test with sera from a patient and naturally infected bandicoot rats showed more than a 4-fold difference. This is in line with data of PRNT investigations of a rat anti-Thai749 immune serum with a large panel of strains of different hantavirus species.[3] Therefore, this report provides additional support for defining THAIV as a distinct species among the hantaviruses.

Schmaljohn et al.[34] reported that the N proteins of HTNV, SEOV, and PUUV have an overall amino acid sequence identity of 50%. However, certain regions of the N protein, such as that spanning amino acid residues 240–310 display only a low level of sequence identity (about 11%) to each other. Therefore, the corresponding N protein-encoding sequence between nt 760–970 is considered as variable region among hantaviruses. By phylogenetic analysis based on nucleotide sequences between positions 375–959 of S genome segment, which contains the variable region, we found a close genetic association of THAIV with the *R. rattus*-associated Cambodian virus strains. Therefore, it is suggested that THAIV and closely related viruses occur throughout Indochina.

The present study extends our knowledge of the geographical distribution and natural host relationships of hantaviruses indigenous to Thailand. A serological survey of rodent samples originating from 22 provinces of Thailand resulted in the identification of hantavirus-reactive samples of *B. indica* from 3 different provinces located in the central plains and northeastern parts (Khorat plateau) of the country. Determination of the endpoint titers of these sera in neutralization assays using HTNV, SEOV, and THAIV revealed infections with THAIV or a THAI-like virus. Similarly, a serum sample originating from *B. savilei* confirmed the occurrence of THAIV or a THAI-like virus in an additional province in the north of Thailand. However, as no viral genetic material is available from *B. savilei* we can not exclude that the detection of THAIV-reactive antibodies is the result of a spill over infection that might have occurred in this region due to a high infectious pressure of this virus. Our findings on the geographical distribution of THAIV overlap with the observations of Nitatpattana et al.,[35,36] who found hantavirus-infected giant bandicoot rats in the central plains as well as in 3 northeastern provinces of Thailand (Khon Kaen, Buri Ram, Surin). A majority of the hantavirus-positive rodents were collected from rice field habitats.[36] In the latter study the highest seroprevalence was observed in giant bandicoot rats from Khon Kaen, an area that lies at the center of the Khorat plateau, whereas comparative quantities of animals collected further east, from Nakhon Phanom and Kalasin, were all hantavirus negative. Unfortunately, we were not able to collect serum samples of bandicoot rats from southern Thailand. Interestingly, a recent serological study conducted in neighboring Cambodia employing HTNV as antigen (660 rodents) found roof rats, Norway rats, and unidentified *Rattus* species infected with hantaviruses closely related to SEOV, but none of 75 bandicoot rats and 183 Polynesian rats (*Rattus exulans*).[32] Therefore, search for THAIV or THAI-like viruses should be extended to southern provinces as well as neighboring Cambodia.[32] THAIV is antigenically cross reactive to HTNV and SEOV. Therefore, previous seroepidemiological studies with the heterologous viruses would detect the prevalence of THAIV infection with the same sensitivity as with THAIV antigen. For further epidemiologic studies, serological typing would certainly profit to elucidate the situation of THAIV infection. Virus isolation and genome amplification from *B. indica* originating from different provinces in Thailand have not been attempted so far, but would be very important to extend our knowledge on the distribution and variability of THAIV and THAI-like viruses in Indochina.

Serological detection of THAIV-reactive antibodies in patients with fever of unknown origin from Surin province confirmed the circulation of THAIV or THAI-like viruses in Thailand. Particularly, results of patient #277 suggest that THAIV or THAIV-like virus causes HFRS. We interpret the close geographical proximity of this THAIV-reactive human sample to the seropositive samples from bandicoot rats as a first indication of a potential epidemiologic relationship. Elwell et al.[4] reported that people living in an area where seropositive giant bandicoot rats were trapped showed a higher seroprevalence than those living in a low prevalence area. Nitatpattana et al.[36] observed that *B. indica* was the species with the highest prevalence of anti-hantavirus antibodies in a study on rodents from northeastern Thailand. Similarly, our study revealed the greater bandicoot rat as the species with the highest seroprevalence against hantavirus in general, and THAIV in particular (3.3% in *B. indica*), and identified the lesser bandicoot rat (*B. savilei*) as a potential new host for THAIV. In combination, this suggests that a higher prevalence of infection of bandicoot rats as such already poses a higher risk for humans to become infected with THAIV than with other hantaviruses from other rodent species. This especially applies to rural areas, where both commensal (e.g., ro-

dent species inhabiting houses) as well as field rodents like bandicoot rats live in close association with humans.[37] In the case of THAIV this is possibly aggravated by the fact that 50–80% of residents in some rural areas trap, cook, and eat *B. indica*.[4,37]

Although a recent publication attributed a first clinical case to hantavirus infection in Thailand,[8] the causative hantavirus species was not further characterized. In our study we identified a patient who developed a clinical profile similar to that of HFRS with high concentrations of IgM and IgG to HTNV by an initial screening of various human sera. Because this serum showed lower titers to HTNV, SEOV, and DOBV antigens compared with virus-specific human positive control sera, and, importantly, contained significant concentrations of virus-neutralizing antibodies against THAIV, these observations suggest that THAIV or a THAI-like virus caused this infection. Furthermore, our FRNT results show close similarities between the particular patient serum and sera from rodents of the genus *Bandicota,* especially *B. indica* representing a host of THAIV.[4] In most hantaviral disease cases, both IgM and IgG to hantavirus are positive at the onset of clinical disease.[38] The reduction of the titer of HTNV-reactive IgM in a follow-up serum sample from convalescent phase of the patient may indicate that THAIV or a related virus is a causative agent of HFRS. However, since hantavirus-reactive IgM might be detected up to 6 months after onset of disease,[39] the possibility that the febrile illness might be caused by infection with other pathogen could not be excluded. Therefore, further epidemiologic study is needed to find out similar patients with hantavirus antibody. Nevertheless, the results of Supputthamongkol et al.[8] and our study indicate that human disease caused by hantaviruses may be more prevalent in Thailand than anticipated earlier, because clinical cases may have been confused with leptospirosis, a rodent-transmitted disease that causes similar symptoms in humans like conjunctival suffusion, hemorrhagic manifestation, renal failure, and hepatic dysfunction.[40] Further epidemiologic studies, including virus isolation, are needed to elucidate the relationship between fevers of unknown origin, presence of THAIV or THAI-like viruses in rodents, and potential transmission from rodents to humans.

In conclusion, we have demonstrated that distribution of *Bandicota*-associated THAIV or THAI-like viruses extends from the central plains of Thailand to the north and northeast. Our genetic and serological studies confirmed the definition of THAIV as a distinct hantavirus species. Moreover, our data suggest that THAIV, besides HTNV and SEOV, may represent an additional causative agent of HFRS in Asia. Recently, we found anti-hantavirus antibody-positive sera both in humans and rodents in Vietnam.[41] Molecular, epidemiologic, and serological studies on hantaviruses in rodents and humans have also been reported from Cambodia and Indonesia.[32,42,] Taken together, this indicates a wide distribution and potentially high diversity of hantaviruses in Southeast Asia calling for further studies on human hantavirus infections, its rodent reservoirs, and possible transmission routes.

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REFERENCES

1. Nichol ST, Beaty BJ, Elliott RM, Goldbach R, Plyusnin A, Schmaliohn CS, Tesh RB, 2005. *Bunyaviridae*. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, eds. *Virus Taxonomy: Classification and Nomenclature of Viruses: Eighth Report of the International Committee on the Taxonomy of Viruses*. San Diego, London, Tokyo: Elsevier Academic Press, 695–716.
2. Carey DE, Reuben R, Panicker KN, Shope RE, Myers RM, 1971. Thottapalayam virus: A presumptive arbovirus isolated from a shrew in India. *Indian J Med Res 59:* 1758–1760.
3. Chu YK, Rossi C, Leduc JW, Lee HW, Schmaljohn CS, Dalrymple JM, 1994. Serological relationships among viruses in the *Hantavirus* genus, family *Bunyaviridae. Virology 198:* 196–204.
4. Elwell MR, Ward GS, Tingpalapong M, LeDuc JW, 1985. Serologic evidence of Hantaan-like virus in rodents and man in Thailand. *Southeast Asian J Trop Med Public Health 16:* 349–354.
5. Xiao SY, Leduc JW, Chu YK, Schmaljohn CS, 1994. Phylogenetic analyses of virus isolates in the genus *Hantavirus*, family *Bunyaviridae. Virology 198:* 205–217.
6. Schmaljohn C, Hjelle B, 1997. Hantaviruses—a global disease problem. *Emerg Infect Dis 3:* 95–104.
7. Marshall JTJ, 1977. Family Muridae. Rat and mice. Lekagul B, McNeely JA, eds. *Mammals of Thailand*. Bangkok: Kurusapha Press, 397–487.
8. Suputthamongkol Y, Nitatpattana N, Chayakulkeeree M, Palabodeewat S, Yoksan S, Gonzalez JP, 2005. Hantavirus infection in Thailand: First clinical case report. *Southeast Asian J Trop Med Public Health 36:* 217–220.
9. Avsic-Zupanic T, Xiao SY, Stojanovic A, Gligic A, van der Froen G, Leduc JW, 1992. Characterization of Dobravavirus: A hantavirus from Slovenia, Yugoslavia. *J Med Virol 38:* 132–137.
10. Nemirov K, Vapalahti O, Lundkvist A, Vasilenko V, Golovljova

I, Plyusnina A, Niemimaa J, Laakkonen J, Henttonen H, Vaheri A, Plyusnin A, 1999. Isolation and characterization of Dobrava hantavirus carried by the striped field mouse (*Apodemus agrarius*) in Estonia. *J Gen Virol 80:* 371–379.
11. Wang H, Yoshimatsu K, Ebihara H, Ogino M, Araki K, Kariwa H, Wang Z, Luo Z, Li D, Hang C, Arikawa J, 2000. Genetic diversity of hantaviruses isolated in china and characterization of novel hantaviruses isolated from *Niviventer confucianus* and *Rattus rattus. Virology 278:* 332–345.
12. Arikawa J, Schmaljohn AL, Dalrymple JM, Schmaljohn CS, 1989. Characterization of Hantaan virus envelope glycoprotein antigenic determinants defined by monoclonal antibodies. *J Gen Virol 70:* 615–624.
13. Yoshimatsu K, Arikawa J, Tamura M, Yoshida R, Lundkvist A, Niklasson B, Kariwa H, Azuma I, 1996. Characterization of the nucleocapsid protein of Hantaan virus strain 76-118 using monoclonal antibodies. *J Gen Virol 77:* 695–704.
14. Arthur RR, Lofts RS, Gomez J, Glass GE, Leduc JW, Childs JE, 1992. Grouping of hantaviruses by small (S) genome segment polymerase chain reaction and amplification of viral RNA from wild-caught rats. *Am J Trop Med Hyg 47:* 210–224.
15. Thompson JD, Higgins DG, Gibson TJ, 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res 22:* 4673–4680.
16. Strimmer K, von Haeseler A, 1997. Likelihood-mapping: A simple method to visualize phylogenetic content of a sequence alignment. *Proc Natl Acad Sci U S A 94:* 6815–6819.
17. Schmidt HA, Strimmer K, Vingron M, von Haeseler A, 2002. TREE-PUZZLE: Maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics 18:* 502–504.
18. Swofford DL, 2002. PAUP* (Phylogenetic analysis using parsimony) (* and other methods), version 4. Sunderland, MA, USA.
19. Yoshimatsu K, Arikawa J, Kariwa H, 1993. Application of a recombinant baculovirus expressing hantavirus nucleocapsid protein as a diagnostic antigen in IFA test: cross reactivities among 3 serotypes of hantavirus which causes hemorrhagic fever with renal syndrome (HFRS). *J Vet Med Sci 55:* 1047–1050.
20. Araki K, Yoshimatsu K, Ogino M, Ebihara H, Lundkvist A, Kariwa H, Takashima I, Arikawa J, 2001. Truncated hantavirus nucleocapsid proteins for serotyping Hantaan, Seoul, and Dobrava hantavirus infections. *J Clin Microbiol 39:* 2397–2404.
21. Corbet GB, Hill JE, 1992. *The Mammals of the Indomalayan Region*. London and Oxford: Natural History Museum Publications and Oxford University Press.
22. Musser GG, Brothers EM, 1984. Identification of bandiccot rats from Thailand (*Bandicota*, Muridae, Rodentia). *Am Mus Novit 3110:* 1–56.
23. Schmidt J, Jandrig B, Klempa B, Yoshimatsu K, Arikawa J, Meisel H, Niedrig M, Pitra C, Krüger DH, Ulrich R, 2005. Nucleocapsid protein of cell culture-adapted Seoul virus strain 80-39: Analysis of its encoding sequence, expression in yeast and immuno-reactivity. *Virus Gene 30:* 37–48.
24. Takakura A, Goto K, Itoh T, Yoshimatsu K, Takashima I, Arikawa J, 2003. Establishment of an Enzyme-linked immunosorbent assay for detection of hantavirus antibody of rats using a recombinant of nucleocapsid protein expressed in Escherichia coli. *Exp Anim 52:* 25–30.
25. Razanskiene A, Schmidt J, Geldmacher A, Ritzi A, Niedrig M, Lundkvist A, Kruger DH, Meisel H, Sasnauskas K, Ulrich R, 2004. High yields of stable and highly pure nucleocapsid proteins of different hantaviruses can be generated in the yeast Saccharomyces cerevisiae. *J Biotechnol 111:* 319–333.
26. Yoshimatsu K, Arikawa J, Yoshida R, Li H, Yoo Y-C, Kariwa K, Hashimoto N, Kakinuma M, Nobunaga T, Azuma I, 1995. Production of recombinant hantavirus nucleocapsid protein expressed in silkworm larvae and its use as a diagnostic antigen in detecting antibodies in serum from infected rats. *Lab Anim Sci 45:* 641–646.
27. Miyamoto H, Kariwa H, Araki K, Lokugamage K, Hayasaka D, Cui BZ, Lokugamage N, Ivanov LI, Mizutani T, Iwasa MA, Yoshimatsu K, Arikawa J, Takashima I, 2003. Serological analysis of hemorrhagic fever with renal syndrome (HFRS) patients in Far Eastern Russia and identification of the causative hantavirus genotype. *Arch Virol 148:* 1543–1556.
28. Ogino M, Ebihara H, Lee BH, Araki K, Lundkvist A, Kawaoka Y, Yoshimatsu K, Arikawa J, 2003. Use of vesicular stomatitis virus pseudotypes bearing Hantaan or Seoul virus envelope proteins in a rapid and safe neutralization test. *Clin Diagn Lab Immunol 10:* 154–160.
29. Plyusnin A, 2002. Genetics of hantaviruses: Implications to taxonomy. *Arch Virol 147:* 665–682.
30. Plyusnin A, Vapalahti O, Lundkvist A, 1996. Hantaviruses: Genome structure, expression and evolution. *J Gen Virol 77:* 2677–2687.
31. Jackson AP, Charleston MA, 2004. A cophylogenetic perspective of RNA-virus evolution. *Mol Biol Evol 21:* 45–57.
32. Reynes JM, Soares JL, Hue T, Bouloy M, Sun S, Kruy SL, Flye Sainte Marie F, Zeller H, 2003. Evidence of the presence of Seoul virus in Cambodia. *Microbes Infect 5:* 769–773.
33. Lokugamage K, Kariwa H, Lokugamage N, Miyamoto H, Iwasa M, Hagiya T, Araki K, Tachi A, Mizutani T, Yoshimatsu K, Arikawa J, Takashima I, 2004. Genetic and antigenic characterization of the Amur virus associated with hemorrhagic fever with renal syndrome. *Virus Res 101:* 127–134.
34. Schmaljohn CS, 1996. Molecular biology of hantaviruses. Elliott RM, ed. *The* Bunyaviridae. New York: Plenum Press, 63–90.
35. Nitatpattana N, Chauvancy G, Dardaine J, Poblap T, Jumronsawat K, Tangkanakul W, Poonsuksombat D, Yoksan S, Gonzalez JP, 2000. Serological study of hantavirus in the rodent population of Nakhon Pathom and Nakhon Ratchasima Provinces Thailand. *Southeast Asian J Trop Med Public Health 31:* 277–282.
36. Nitatpattana N, Henrich T, Palabodeewat S, Tangkanakul W, Poonsuksombat D, Chauvancy G, Barbazan P, Yoksan S, Gonzalez JP, 2002. Hantaan virus antibody prevalence in rodent populations of several provinces of northeastern Thailand. *Trop Med Int Health 7:* 840–845.
37. Boonsong P, Hongnark S, Suasa-ard K, Khoprasert Y, Promkerd P, Hamarit G, Nookarn P, Jäkel T, 1999. Rodent management in Thailand. Singleton G, Leirs H, Hinds L, Zhang Z, eds. *Ecologically-Based Rodent Management*. Camberra: Australian Center for International Agricultural Research, 338–357.
38. Rossi C, Ksiazek T, 1999. Virus Detection and identification with serological tests: 2. Enzyme-linked immunosorbent assay (ELISA). Lee HW, Calisher C, Schmaljohn CS, eds. *Manual of Hemorrhagic Fever with Renal Syndrome and Hantavirus Pulmonary Syndrome*. Seoul, Korea: WHO Collaborating Center for Virus Reference and Research, 87–91.
39. Elgh F, Wadell G, Juto P, 1995. Comparison of the kinetics of Puumala virus specific IgM and IgG antibody responses in nephropathia epidemica as measured by a recombinant antigen-based enzyme-linked immunosorbent assay and an immunofluorescence test. *J Med Virol 45:* 146–150.
40. Levett PN, 2001. Leptospirosis. *Clin Microbiol Rev 14:* 296–326.
41. Truong T-T, Truong U-N, Yoshimatsu K, Lee B-H, Araki K, Arikawa J, 2004. Report of Serology Hantavirus in Human and Rodent at Vietnam in 2003. The 6th International Conference on Hemorrhagic Fever with Renal Syndrome (HFRS), Hantavirus Pulmonary Syndrome (HPS) and Hantaviruses. Seoul, Korea, 146.
42. Plyusnina A, Ibrahim IN, Winoto I, Porter KR, Gotama IB, Lundkvist A, Vaheri A, Plyusnin A, 2004. Identification of Seoul hantavirus in *Rattus norvegicus* in Indonesia. *Scand J Infect Dis 36:* 356–359.