Epidemiology of Hantavirus Infection in Thousand Islands Regency of Jakarta, Indonesia

Ima-Nurisa IBRAHIM1), Kenta SHIMIZU2), Kumiko YOSHIMATSU2), Andre YUNIANTO1), Ervi SALWATI1), Shumpei P. YASUDA2), Takaaki KOMA2), Kumiko YOSHIMATSU2), Andre YUNIANTO1), Ervi SALWATI1), Shumpei P. YASUDA2), Takaaki KOMA2), Rika ENDO2) and Jiro ARIKAWA2)*

1)National Institute of Health Research and Development, Ministry of Health, Jl. Percetakan Negra 29, Jakarta 10560, Indonesia
2)Department of Microbiology, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060–8638, Japan

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ABSTRACT. Hemorrhagic fever with renal syndrome (HFRS) is a rodent-borne zoonotic disease caused by hantavirus infection. Many HFRS cases have been reported in East Asia and North Europe, while the situation in Southeast Asia remains unclear. In this study, the prevalence of hantavirus infection in rodents and humans in Thousand Islands regency, which is close to the port of Jakarta, one of the largest historic ports in Indonesia, was investigated. A total of 170 rodents were captured in 2005, and 27 (15.9%) of the rodents were antibody-positive against Hantaan virus antigen in an immunofluorescence assay (IFA) and Western blotting. Despite the high prevalence in rodents, human sera collected from 31 patients with fever of unknown origin and 20 healthy volunteers in the islands in 2009 did not show positive reaction to the antigen in IFA. To identify the virus in rodents genetically, a total of 59 rodents were captured in 2009. Sera from the rodents were screened for antibody by ELISA, and lung tissues were subjected to RT-PCR. 20 (33.9%) of the 59 rodents were antibody-positive, and 3 of those 20 rodents were positive for S and M genome segments of hantaviruses. Genetic analysis showed that the viruses belonged to Seoul virus and formed a cluster with those in Vietnam and Singapore. These results suggest that a unique group of Seoul viruses has spread widely in Southeast Asia.

KEYWORDS: epidemiology, hantavirus, Indonesia, rodent, Seoul virus.
were identified by outer appearance and sizes of body parts. Sera were collected from all of the rodents, while lung tissues were collected from rodents captured in 2009. A total of 51 human sera were collected from 31 patients with fever of unknown origin and from 20 healthy volunteers at community health centers in Tidung and Panggang Islands from June 2009 to October 2009 (Table 4).

**Immunofluorescence assay (IFA):** Rodent sera collected in 2005 and human sera were screened for antibodies against hantaviruses by IFA as reported previously [23]. Hantaan virus (HTNV)-infected Vero E6 cells were used for the antigen. Sera were examined at 1:200 dilutions. Fluorescein isothiocyanate (FITC)-conjugated goat anti-rat IgG (KPL) and Protein A-FITC conjugate (EY-lab) were used for rodent and human sera, respectively. The specific granular pattern of fluorescence in cytoplasm was examined under a fluorescence microscope.

**Western blotting (WB):** After screening by IFA, the presence of antibodies against hantaviruses was confirmed by WB as reported previously [24]. Recombinant HTNV N expressed by a baculovirus system was used for the antigen.
Horseradish peroxidase (HRP)-conjugated goat anti-rat IgG (KPL) and 4-chloro-1-naphthol (Wako) were used for the secondary antibody and substrate, respectively.

Enzyme-linked immunosorbent assay (ELISA): Rodent sera collected in 2009 were screened for antibodies against hantaviruses by ELISA as reported previously [14]. Briefly, 96-well EIA/RIA plates (Corning) were coated with bacterially-expressed HTNV N at 4°C overnight. After blocking with PBS(−) containing 3% bovine serum albumin (Sigma-Aldrich, St. Louis, MO, U.S.A.), the plates were incubated with 1:200 dilutions of heat-inactivated sera at room temperature for 1 hr. After washing with PBS(−) containing 0.05% Tween 20 three times, the plates were incubated with HRP-conjugated goat anti-rat IgG (KPL) at room temperature for 1 hr. After washing and coloring with o-phenylenediamine (Sigma), optical density (OD) at a wavelength of 450 nm was measured by a spectrometer. Cutoff value was set at OD=0.864, which was the minimum OD value in ELISA among the antibody-positive sera collected in 2005.

Sequence analysis of hantavirus genome: Total RNA isolated from rat lung tissue was used to examine hantavirus-specific cDNA as described previously [22]. Briefly, total RNAs were extracted from lung homogenate of antibody-positive rodents with ISOGEN (Nippon Gene, Tokyo, Japan) and reverse-transcribed into cDNAs by using random hexamer and SuperScript II reverse transcriptase (Life Technologies Japan Ltd., Tokyo, Japan) according to the manufacturer’s instructions. cDNAs were amplified by polymerase chain reaction (PCR) by using AmpliTaq Gold (Life Technologies Japan Ltd.) and primers specific for hantaviruses (Table 1). PCR products were purified with a MinElute PCR purification kit (QIAGEN GmbH, Hilden, Germany) and subjected to nucleotide sequencing with a BigDye Terminator v3.1 Cycle Sequencing Kit and ABI PRISM 3130xl Genetic Analyzer (ABI) according to the manufacturer’s instructions. Phylogenetic analysis was performed by using GENETYX-MAC (ver. 15.0.1), CLUSTAL W (ver.1.83) and Njprot (ver. 2.3).

RESULTS

Prevalence of hantavirus infection in rodents captured in 2005: A total of 170 rodents (74 R. norvegicus and 96 R. tanezumi) were captured in six islands in Thousand Islands regency, and 27 (15.9%) of the rodents were positive for antibodies against hantaviruses in IFA and WB (Table 2). Seroprevalences in R. norvegicus ranged from 17.2 to 40.6% (28.4% in total), while those in R. tanezumi ranged from 0 to 20.8% (6.3% in total). Antibodies were not detected from rodents in Pari, Kotok and Rambut Islands.

Screening of antibodies in rodents captured in 2009: Genetic identification of the virus in the rodents captured in 2005 was hampered by lack of samples feasible for genetic examination. Hence, rodents were captured again in 2009, and sera as well as lung tissues were collected for subsequent genetic examination. A total of 59 rodents (38 R. norvegicus and 21 R. tanezumi) were captured in three islands, and 20 (33.9%) of the rodents were antibody-positive in ELISA (Table 3). Similar to the results for rodents captured in 2005, seroprevalences in R. norvegicus were higher than those in R. tanezumi (33.3–46.2% vs. 0–20.0%). Seroprevalences in rodents in Tidung and Panggang Islands were continuously high in both 2005 and 2009.

Detection and sequence analysis of hantavirus genome: 20 lung tissues of the antibody-positive rodents captured in 2009 were examined for the presence of hantavirus genome by RT-PCR. We chose three specimens, such as KS74, KS80 and KS90, for the sequencing analysis, because they showed clear amplification patterns. cDNA fragments derived from S and M genome segments were amplified from three lung tissues of rodents: one R. norvegicus rodent captured in Panggang Island (KS90) and two R. norvegicus rodents captured in Tidung Island (KS74 and KS80). Partial sequences of the S segment (129 to 1,570 nucleotides) and the M segment (1,971 to 3,124 nucleotides) were determined. As for the S segment of KS90, the sequence of a shorter region (839 to 1,570 nucleotides) was determined. Alignment and phylogenetic analysis with known sequences showed that the viruses belonged to SEOV and formed a cluster with those detected in Vietnam and Singapore (Fig. 2) [9, 12, 21].

Prevalence of hantavirus infection in residents of the islands: To estimate the risk of hantavirus infection in residents, 31 sera of patients with fever of unknown origin and 20 sera of healthy volunteers in Tidung and Panggang Islands were screened for antibodies against hantaviruses by IFA (Table 4). Despite the relatively high prevalence in rodents, there were no antibody-positive sera in either patients or healthy volunteers.

DISCUSSION

Hantavirus-infected rodents have been reported in Southeast Asian countries including Vietnam, Cambodia, Thailand and Singapore [2, 9, 12, 18, 21]. Antibody positivity among humans has also been reported in Asian countries including India, Sri Lanka and Vietnam [3, 5, 7]. In the present study, we found SEOV-positive rodents in Thousand Islands regency of Jakarta in Java Sea, where many ships traffic frequently. In addition, phylogenetic analysis showed that Indonesian SEOV belongs to the same lineage as that of SEOV in Vietnamese and Singapore. These results suggest that SEOV was transported through movement of people accompanied by infected rodents beyond the Java Sea and that a unique group of SEOV spread widely in Southeast Asia. Generally, hantaviruses are thought to have co-evolved with host rodents [16]. Indonesia is thought to be the area.
where original Rattus rodents appeared and spread to other areas. Therefore, Indonesia is inhabited by many unique rats [1]. Thus, not only SEOV but also various other types of hantaviruses might exist in many unique rodents in Indonesia and spread to Southeast Asian countries. In fact, after the discovery of SERV in Indonesia [15], SERV-like viruses were also discovered in Singapore [9]. In this paper, we failed to detect hantaviruses from R. tanezumi captured in Thousand Island district. Because antibody prevalence rate among R. tanezumi was lower than R. norvegicus and viral genome was not detected from any them, spillover infection of R. norvegicus-borne SEOV to R. tanezumi is suggested.

It was very similar to the situation of SEOV infection in Haiphong port, Vietnam [22]. In addition to the difficulties of virus detection, taxonomic classification of R. tanezumi in Asia has also not yet been fixed [1]. Furthermore, genetical comparison between R. tanezumi in Serang district and Thousand Island district has not been completed. To understand situation of Rattus-borne hantaviures in nature, additional informations of hantaviruses and host animals are required.
in future.

Prevalences of hantavirus infection in rodents were higher in populated islands, such as Tidung, Panggang and Untung Jawa Islands. Hantaviruses might have been easily introduced and maintained in these populated islands, because rodents have an abundant food supply from garbage and can reproduce efficiently. Indeed, the prevalence of hantavirus infection in rodents in Tidung and Panggang Islands in 2009 was continuously high as that in 2005.

Despite the relatively high prevalence in rodents in Tidung and Panggang Islands, antibodies against hantaviruses were not detected from residents of these islands, implying that the risk of hantavirus infection in humans may not be high in those islands. However, it is important to try to avoid direct and indirect contacts with rodents and improve hygiene, because rodents have a number of pathogens.

All of the rodents captured in Thousand Islands were infested with at least one group of ectoparasites (fleas, lice, tick or mites). The only species of flea found in this study was Xenopsylla cheopis, which is known as a vector of plague, and the flea index (average number of fleas per host) was 2.8 for R. norvegicus and 0.4 for R. tanezumi. It has been reported that a flea index of greater than 1 for X. cheopis on rats represents a potentially dangerous situation with respect to increased plague risk for humans [4]. These observations suggest that the potential risk for outbreaks of plague as well as other rodent-borne diseases in the district is high. Continuous investigation in this area is needed to control rodent-borne diseases and improve public health.

We found SEOV-infected rodents in tiny islands, the perimeters of which are as small as several kilometers. The isolated nature and small sizes of these islands are suitable for monitoring the pathogen and reservoirs, evaluating the outcome of countermeasures and studying the ecology of hantaviruses in nature.

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