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研究報告の概要

ループス腎炎による末期腎不全の 23 歳の男性が、生体腎移植を受けた。生体腎移植のドナーである母親には 6 カ月前にデング熱の既往があった。男性患者は術後 5 日目に 39℃発熱した。他に症状はなく、感染源は不明であった。ドナーのデング熱既往歴を鑑み、デングウイルスの RT-PCR 検査を行なった結果、男性患者は陽性であった。ウイルス血症の明らかな回復後も、ドナーの臓器がデングウイルスに感染したままだったかどうか不明である。蚊の媒介による 2 次感染があったかもしれないが、この時期、この地域でデングの活発な伝播はみられず、病院での感染の可能性も少ない。
デング流行地域での臓器や血液製剤のドナースクリーニングは、その感度、実行性、コストベネフィットの評価が必要であるものの有益であるかもしれない。

報告企業の意見	今後の対応
デング熱既往があるドナーからの生体腎移植により、デングウイルスに感染した可能性を示唆した報告である。血漿分画製剤からのデングウイルス伝播事例は報告されていない。また、万一原料血漿にデングウイルスが混入したとしても、BVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から抗D（Rho）人免疫グロブリンを濃縮・精製した製剤であり、ウイルス除去を目的として、製造工程において濾過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。

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Nephrology Dialysis Transplantation

Case Report

Dengue haemorrhagic fever after living donor renal transplantation

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Introduction

The transmission of infection from donor to recipient in solid organ transplantation can result in loss of the allograft and in severe cases, death of the recipient. The occurrence of dengue virus infection in an immunocompromised renal transplant patient can have many detrimental effects, the most life-threatening of these is development of dengue shock syndrome. We present a case of possible transmission of dengue infection from donor to recipient after living donor renal transplantation, resulting in a fulminant course of dengue haemorrhagic fever (DHF).

Case report

A 23-year-old male with end-stage renal failure due to lupus nephritis underwent a living donor renal transplantation. The donor was his mother, who was healthy except for a significant past history of dengue fever 6 months prior. Early post-operative recovery was uncomplicated with immediate graft function and serum creatinine of 67 μmol/l on post-operative day 2. His immunosuppressive medication consisted of mycophenolate mofetil, tacrolimus and methylprednisolone.

On the 5th post-operative day, he spiked a temperature of 39°C. He was asymptomatic and physical examination did not reveal a source of infection. In view of a previous history of dengue infection in the donor, blood was analysed for dengue virus using real-time polymerase chain reaction (RT-PCR). This was positive for Dengue virus Serotype 1.

Over the next week, he continued to spike high temperatures with a fall in platelet count and a deterioration of his general clinical condition. On the 12th post-operative day, he developed upper gastrointestinal bleeding, gross haematuria and tachycardia. Investigations revealed that his haemoglobin had dropped to 4.6 g/dl, leucocyte count was 0.86×10^9/l, platelet count was 71×10^9/l, serum lactate dehydrogenase was 1322 U/l (normal: 300–700 U/l) and albumin was 14 g/l (normal: 38–49 g/l). His mycophenolate mofetil dose was discontinued and granulocyte-colony stimulating factor was commenced for his profound leucopenia. He required multiple packed cell and platelet transfusions. Chest X-ray revealed a right sided pleural effusion. On the 15th post-operative day, he complained of left flank pain and abdominal distension. One litre of blood was drained from his Tenckoff catheter. Computed tomography of his abdomen revealed a large retroperitoneal haematoma arising from the bed of the transplanted kidney. Emergency laparotomy was undertaken for surgical haemostasis and evacuation of the haematoma. Intra-operatively, 1.5 l of blood was drained from the retroperitoneal space. A generalized ooze was seen from the tissue bed of the allograft and this was treated with packing. There was a perforation seen in the peritoneum, which allowed communication between the retroperitoneal and intraperitoneal spaces. The transplanted kidney appeared healthy. A repeat dengue RT-PCR was negative.

Post-operative recovery was uneventful with resolution of fever and recovery of thrombocytopenia within the next week. Haemorrhagic tendencies ceased spontaneously with resolution of haemetemesis and haematuria. His graft function remained excellent. Figure 1 depicts the course and progression of his illness.

Discussion

The pathophysiological mechanisms underlying DHF remains controversial. There is strong evidence to suggest an increased risk of DHF with secondary dengue virus infection [1]. The presence of pre-exisiting antidengue antibodies increases viraemia titres by a phenomenon known as antibody-dependent enhancement of infection (ADE) [2].

Having lived in a dengue-endemic region, our patient may have been infected previously but was asymptomatic or had subclinical infection. A large proportion of the adult population in Singapore have been exposed to dengue as reflected by a high prevalence of

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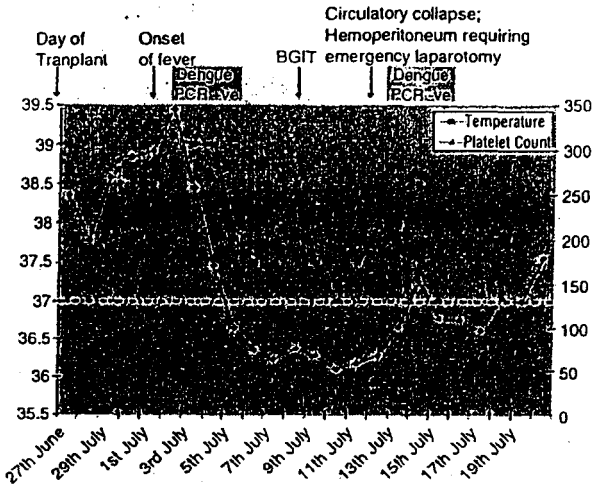

Fig. 1. Trend of temperature and platelet count correlated to clinical findings during period of illness. Dotted line represents normal values: normal body temperature taken as 37°C; lower limit of normal for platelet count taken as 130×10^9 g/l. PCR, polymerase chain reaction; BGIT, bleeding gastrointestinal tract. Minus sign indicates negative results, plus sign indicates positive result.

dengue seropositivity (45%) [3]. Transplantation of the dengue-infected allograft can cause secondary infection and development of DHF. It is not known whether donor organs remain infected after apparent resolution of viraemia. Alternatively, secondary transmission could have been via the usual route, the bite of a mosquito. However, there was no active transmission of dengue in our area during this period, making hospital-acquired infection less likely.

Clinical presentation and course of the illness in this immunosuppressed patient is similar to that in the immunocompetent, except for a longer period of illness. Our patient experienced a prolonged course of illness (19 days) as well as prolonged duration of thrombocytopenia (12 days). The mean duration of illness is 2–7 days and duration of thrombocytopenia 3.6 days (±1.6 days) [4]. The critical stage in DHF is usually around the time of defervescence, with circulatory failure of haemorrhagic manifestations occurring about 24 h before to 24 h after the temperature falls to normal or below. This was the case for our patient with shock and haemoperitoneum occurring on the same day as defervescence of fever (Figure 1).

DHF occurring early post-operatively poses a potential danger to the transplant patient. Bleeding diathesis resulting from thrombocytopenia, dysfunctional surviving platelets and increased fibrinolysis result in persistent haemorrhage especially from cut tissue surfaces. In our patient, profuse, persistent bleeding from the tissue bed of the transplanted kidney led to circulatory collapse and a need for surgical drainage of haematoma and haemostasis. In addition, he also had other haemorrhagic manifestations with gastrointestinal bleeding and haematuria. Hypovolaemia poses a risk of damage to the allograft. Hypoalbuminaemia secondary to leakage of plasma aggravates the problem of poor wound healing in the immunosuppressed transplant recipient. No specific therapy or vaccine exists for DHF. Management is supportive, with correction of hypovolaemia and coagulation abnormalities.

This case illustrates the severity of DHF after renal transplantation of an infected allograft. In dengue-endemic regions, clinicians should have a high index of suspicion for DHF in patients with viral haemorrhagic fevers. Screening donors of organs and blood products may be beneficial, although sensitivity, feasibility and cost benefits of screening need to be assessed.

Conflict of interest statement. None declared.

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一般的名称	乾燥抗D（Rho）人免疫グロブリン	研究報告の公表状況	Microbiology and Immunology, 48(11), 2004	公表国 日本
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研究報告の概要

日本における、腎症候性出血熱のアウトブレイクは1960年代以降2回あった。1回目は1960年代の大阪市の梅田地区で起こり、その原因は都市型ネズミ（ドブネズミ）であった。2回目は1970年から1984年の間に様々な動物施設で報告され、実験室のラット（ドブネズミ）からヒトへの感染であった。1985年以降現在まで、腎症候性出血熱の事例は報告されていない。
2000年～2003年に日本各地において齧歯類のハンタウイルス感染について疫学調査を行った。合計1,221匹の齧歯動物と食虫動物が捕獲された。血清陽性の動物は、アカネズミ（5/482、1.0%）、ドブネズミ（4/364、1.1%）、クマネズミ（3/45、6.7%）とエゾヤチネズミ（7/197、3.6%）で見つかった。函館で捕獲された血清陽性のクマネズミの1匹から5セグメントの部分が増幅され、この核酸配列はソウルウイルス（SEOV）原型株 SR-11 と 96%の相同性を示した。更に高リスク集団における人ハンタウイルス感染の疫学調査により、北海道の陸上自衛隊員207人のうち1人がIFA、ELISA及びWB分析で、抗ハンタウイルス抗体陽性であり、血清型特異的ELIZAの結果からソウルウイルス（SEOV）感染が示唆された。人の SEOV の感染源は港湾地区の感染したクマネズミやドブネズミであり、旅行者や港湾労働者にとっては脅威となるかもしれない。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から抗D（Rho）人免疫グロブリンを濃縮・精製した製剤であり、ウイルス除去を目的として、製造工程において濾過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見

2000年～2003年の日本におけるハンタウイルス感染に関する動物の疫学調査の結果、北海道における人での疫学調査で1名ソウルウイルス感染が示唆されたとする報告である。
血漿分画製剤からのハンタウイルス伝播の事例は報告されていない。また、万一原料血漿にハンタウイルスが混入したとしても、BVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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Epizootiological and Epidemiological Study of Hantavirus Infection in Japan

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Abstract: **Epizootiological surveys on hantavirus infections in rodents were carried out in various areas of Japan, including the four major islands of Hokkaido, Honshu, Shikoku, and Kyushu from 2000 to 2003. A total of 1,221 rodents and insectivores were captured. Seropositive animals were found in *Apodemus* (*A.*) *speciosus* (5/482, 1.0%), *Rattus* (*R.*) *norvegicus* (4/364, 1.1%), *R. rattus* (3/45, 6.7%), and *Clethrionomys* (*C.*) *rufocanus* (7/197, 3.6%). The partial S segment was amplified from one seropositive *R. rattus* captured at Hakodate. The nucleotide sequence showed 96% identity with the Seoul virus (SEOV) prototype strain SR-11. In addition, we conducted an epidemiological survey on human hantavirus infection in a high-risk population, the personnel of the Japan Ground Self-defense Force on Hokkaido. One out of 207 human blood samples was positive for anti-hantavirus antibody by IFA, ELISA, and WB analysis. The result of the serotype specific ELISA indicates that this individual acquired SEOV infection. This study indicates that *A. speciosus*, *R. norvegicus*, *R. rattus*, and *C. rufocanus* carry hantaviruses as the reservoir animals in Japan. Infected *R. rattus* and *R. norvegicus* in port areas could be the sources of human SEOV infection and a threat to travelers and individuals working in seaports.**

Key words: Hantavirus, Rodent, Epidemiology, Epizootiology

Hantaviruses are causative agents of two human illnesses, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). The animal reservoirs of hantaviruses are various rodent species, which, when infected, do not show any symptoms and carry the virus for long periods. Humans acquire hantavirus infection by inhalation of virus-containing excreta from infected animals. More than 20 serotypes or genotypes of hantaviruses have been reported, and each virus has a specific rodent reservoir. Because the phylogenies of the viruses and the reservoir rodents are topologically identical, it is generally believed that hantaviruses and rodents have co-evolved (17). The factors responsible for the emergence of human hantavirus infections include changes in ecological factors and changes in human activities. The most important risk factor is close contact with rodents as a result of agricultural, forestry, or military activities (14).

HFRS is caused by Hantaan virus (HTNV), Seoul virus (SEOV), Puumala virus (PUUV), and Dobrava virus (DOBV) in Eurasia; these viruses are carried by

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Abbreviations: AMRV, Amur virus; ANDV, Andes virus; BAYV, Bayou virus; BCCV, Black Creek Canal virus; CMC, carboxymethyl cellulose; DOBV, Dobrava virus; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; FFU, focus forming unit; FITC, fluorescein isothiocyanate; FRNT, focus reduction neutralization test; HFRS, hemorrhagic fever with renal syndrome; HPS, hantavirus pulmonary syndrome; HTNV, Hantaan virus; IFA, indirect immunofluorescent-antibody assay; MEM, minimum essential medium; NP, nucleocapsid protein; NYV, New York virus; OD, optical density; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PUUV, Puumala virus; PVDF, polyvinylidene fluoride; SAAV, Saaremaa virus; SEOV, Seoul virus; SNV, Sin Nombre virus; WB, Western blot.

Apodemus (*A.*) *agrarius*, *Rattus* (*R.*) *norvegicus* and *R. rattus*, *Clethrionomys* (*C.*) *glareolus*, and *A. flavicollis*, respectively (3). Recently, Saaremaa virus (SAAV) was identified as the causative agent of a mild form of HFRS in Europe, and *A. agrarius* was found to be the carrier (15, 18). There is serological evidence of human SAAV infections in Estonia (4), and SAAV might have been the cause of an HFRS outbreak in Russia in the 1990 s (18).

On the American continent, HPS is caused by Sin Nombre virus (SNV), New York virus (NYV), Black Creek Canal virus (BCCV), Bayou virus (BAYV), and Andes virus (ANDV), which are carried by *Peromyscus* (*P.*) *maniculatus*, *P. leucopus*, *Sigmodon hispidus*, *Oryzomys palustris*, and *Oligoryzomys longicaudatus*, respectively (16, 17).

About 200,000 HFRS cases are reported annually throughout the world (10). A wide variety of hantaviruses responsible for HFRS have been found in East Asia (19). About 50,000 to 100,000 HFRS cases are reported annually in China, where HTNV and SEOV are responsible for most of the cases (20). In addition, Far East Russia is well known as an endemic area for HFRS. About 100 to 200 HFRS patients are reported annually in that region. Recently, a distinct type of hantavirus, Amur virus (AMRV), was identified in HFRS patients in Far East Russia (23). Our previous studies revealed that *A. peninsulae* is the reservoir animal for AMRV, as well as revealing antigenic and genetic evidence of a distinct hantavirus serotype (12, 13). Furthermore, Khabarovsk and Vladivostock viruses were also identified in the same region (5, 6).

In Japan, there have been two outbreaks of HFRS since the 1960s. One was reported in the Umeda district in Osaka city in the 1960s (22). The source of the infection is believed to have been urban rats (*R. norvegicus*). The other outbreak was reported in various animal facilities in the country between 1970 and 1984, and the human infections were related to contact with laboratory rats (*R. norvegicus*) (9, 11). Since 1985 to the present, there have been no reported HFRS cases. However, seropositive *R. norvegicus* have been identified in ports and reclaimed areas in different locations throughout the country (2). In addition, Puumala-related viruses are widely distributed in *C. rufocanus* on Hokkaido, the northern-most major island of Japan (6, 8).

Although our recent study identified anti-hantavirus antibodies among patients with hepatitis of unknown etiology in Japan, the prevalence of the antibody is very low in the general population (7). It is unclear why Japan has few HFRS patients in spite of the highly endemic nature of the disease in surrounding countries. To clarify this question, a large-scale epizootiological study targeting indigenous rodents was essential. Therefore, we carried out epizootiological surveys in rodents from various areas of Japan, including the four major islands of Hokkaido, Honshu, Shikoku, and Kyushu, to determine the endemic areas and the reservoir animals.

Materials and Methods

Cells and viruses. Hantavirus strains HTN 76-118, SR-11, and Sotkamo, were propagated as representative strains of HTNV, SEOV, and PUUV, respectively, in Vero E6 cells grown in Eagle's minimum essential medium (MEM) supplemented with 5% fetal bovine serum (FCS). The cultured medium of the infected cells was harvested 7–14 days later and stored as stock virus at −80 C. All experiments with live viruses were carried out in a P3 containment room.

Rodent sera. A total of 1,221 rodent and insectivore sera were collected from field surveys on Hokkaido, Honshu, Shikoku, Kyushu, and Tsushima Island in Japan from 2000 to 2003. The species captured were *C. rufocanus*, *C. rutilus*, *A. speciosus*, *A. argentius*, *R. norvegicus*, *R. rattus*, *M. montebelli*, *E. smithi*, *M. minutus*, *M. musculus*, *C. dsinezumi*, and *U. talpoides*. Blood samples were collected from live animals by cardiac puncture under anesthesia. Blood samples from dead animals were collected on filter papers (Toyo, Tokyo). After sampling, the papers with absorbed blood were air-dried, cut into four pieces, and collected into microcentrifuge tubes containing 0.4 ml of phosphate buffered saline (PBS). The tubes were held at 4 C over night and then heated at 56 C for 30 min. The tubes were centrifuged at 10,000 revolutions per minitue for 10 min and the supernatants were transferred to new tubes as 1:10 diluted sera. All collected sera were stored at −40 C until use.

Human sera. A total of 207 human sera were collected from the personnel of the Japan Ground Self-defense Force, who had been training on Hokkaido for several years. The blood samples were processed as described above for the rodent samples and stored at −40 C until use.

Indirect immunofluorescent antibody assay (IFA). Vero E6 cells in 75-cm^2 flasks were infected with HTNV, SEOV, or PUUV and cultured for 6, 7, or 11 days, respectively. The cells were collected by trypsinization and seeded onto 24-well slides. The slides were incubated for 4 hr at 37 C in a CO_2 incubator. The cells were fixed with cold acetone for 20 min and air dried for 1 hr. The prepared slides were stored at −40 C until use. The sera were spotted onto the 24-

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well slides and incubated for 1 hr at 37 C. The slides were washed with PBS and spotted with fluorescein isothiocyanate (FITC)-conjugated protein G (Zymed, Laboratories, Inc., San Francisco, Calif., U.S.A.). After incubation at 37 C for 1 hr, the slides were washed and examined under fluorescence microscopy. Scattered and granular fluorescence in the cytoplasm of infected cells was considered a positive reaction.

Focus reduction neutralization test (FRNT). Serially diluted rodent sera (50 µl) were mixed with an equal volume of stock virus, either HTNV or SEOV, containing 200 focus forming units (FFU)/50 µl and incubated at 37 C for 1 hr in a CO_2 incubator. The mixtures were then inoculated onto Vero E6 cell monolayers grown on 8-chamber slides. The slides were incubated at 37 C for 1 hr, and the inoculum was removed. The cells were overlayed with MEM (supplemented with 5% FCS) containing 1.5% carboxymethyl cellulose (CMC) and incubated in a CO_2 incubator at 37 C for 6 to 11 days. After incubation, the infected cells were washed with PBS four times, fixed with methanol, and air-dried. To visualize the foci of virus-infected cells, IFA was carried out. Mouse immune serum to HTNV or SEOV was added to the Vero E6 cells on the slides inoculated with HTNV or SEOV, respectively. After incubation for 1 hr at 37 C and three washes with PBS, FITC-conjugated antibody to mouse IgG (ICN Pharmaceuticals, Inc., Aurora, Ohio, U.S.A.) was applied. After incubation for 1 hr at 37 C, the foci were counted under a fluorescence microscope. The FRNT titer was defined as the highest dilution of the serum that reduced the number of foci by at least 80%.

Western blot (WB). Western blot was performed using the recombinant nucleocapsid protein (rNP) of PUUV as an antigen (Kariwa et al., in preparation for publication). The rNP of PUUV was separated by sodium dodecyl sulfate-12% polyacrylamide gel electrophoresis and blotted onto polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, Mass., U.S.A.). Mouse immune serum to PUUV was used as a positive control to detect antigen on the membrane. IFA-positive rodent sera were reacted with the membrane, and bound antibodies were detected with horseradish peroxidase-labeled protein G (Prozyme, Inc., Calif., U.S.A.). The peroxidase substrate, 4-chloro-1-naphthol, was used to visualize the bands.

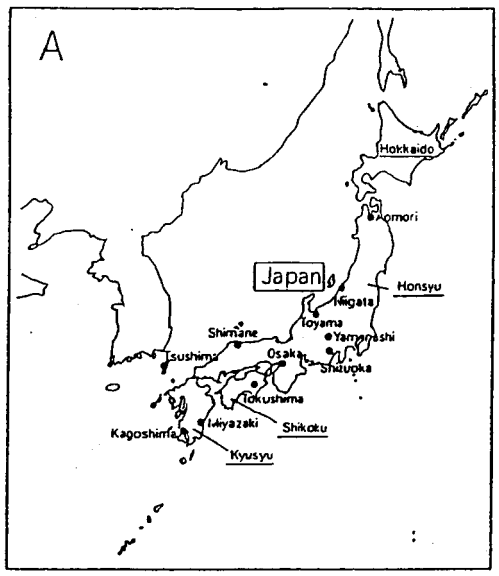
Capture enzyme-linked immunosorbent assay (ELISA). Seropositive human sera from the individuals tested at the Japan Ground Self-defense Force were further examined by capture-ELISA, using baculovirus-expressed recombinant HTNV, SEOV, and DOBV nucleocapsid proteins (rNP) produced in insect cells (1). The Fab region of mouse monoclonal antibody E5/G6 was used as the capture antibody to exclude non-specific reactions. Ninety-six-well plates were coated with E5/G6 (2 µg/ml in PBS) at 4 C overnight. The plates were then incubated with the baculovirus-expressed rNP at 37 C for 1 hr. As a negative control antigen, we used Borna disease virus p24 expressed by the baculovirus system. Each well was then incubated with 1:400-diluted seropositive serum samples or seronegative sera (negative control) at 37 C for 1 hr. After incubation, goat anti-human IgG conjugated with alkaline phosphatase was applied to the wells and incubated at 37 C for 1 hr. Finally, pNPP substrate was added, the plate was held at room temperature for 30 min, and the optical density (OD) was measured at 405 nm. OD values exceeding the mean of the serum control wells plus twice the standard deviation were regarded as positive.

Polymerase chain reaction (PCR) and sequence analysis. Total RNA was extracted from the lungs of seropositive *R. rattus* and *R. norvegicus* with Isogen reagent (Nippon Gene Co., Ltd., Osaka, Japan), according to the manufacturer's instructions. cDNA was synthesized using 5 µg of total RNA, Superscript II (200 U), and random primer (Invitrogen, Carlsbad, Calif., U.S.A.) incubated at 42 C for 50 min and at 70 C for 15 min. Partial S segments were amplified using the primer pair SEO-66 (5'-GAGAGAAATCAGTGCTCACG-3') and SEO-801 (5'-ATAAACTCCCGGCAATAAGA -3') and further amplified by an inner primer pair specific for SEOV, SEO-96 (5'-TGTGATAGCACGCCAGAAGG-3') and SEO-542 (5'-TCCTCATATGAGCTGTCATC-3'). The polymerase chain reaction (PCR) program consisted of 35 cycles of denaturation at 94 C for 30 sec, annealing at 52 C for 30 sec, and extension at 68 C for 2 min. The amplified S segments were sequenced directly using a Big Dye terminator (Applied Biosystems, Foster City, Calif., U.S.A.) and an ABI 310 Genetic Analyzer.

Results

Epizootiological studies of hantavirus infection among wild rodents were conducted in various locations of Japan including the 4 major islands, i.e., Honshu, Kyushu, Shikoku, and Hokkaido from 2000 to 2003.

A total of 806 rodents and insectivores were captured from 11 wild settings on Honshu, Shikoku, Kyushu, and Tsushima Islands and from six sites on Hokkaido. Figure 1 shows the geographical locations of the survey sites. The sera were screened by IFA for anti-hantavirus antibodies. A total of 592 rodent sera from wild settings in the southern regions of Japan, including



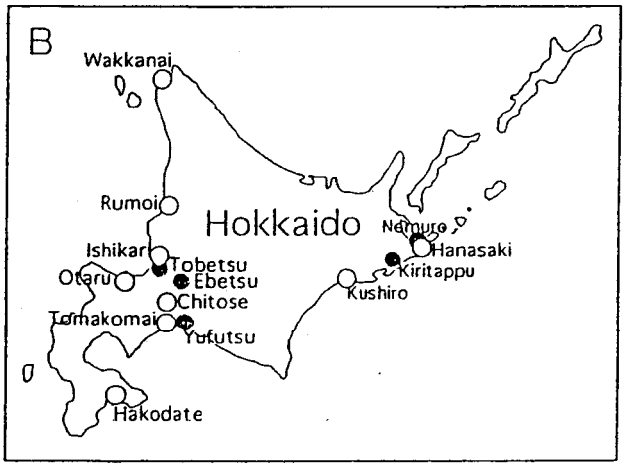


Fig. 1. Geographical location of epizootiological survey sites. (1) Surveys were carried out in Aomori, Niigata, Toyama, Yamanashi, Shizuoka, Osaka, Tokushima, Shimane, Tsushima, Miyazaki, and Kagoshima prefectures. (2) Surveys were carried out in Wakkanai, Rumoi, Ishikari, Otaru, Tomakomai, Hakodate, Tobetsu, Ebetsu, Chitose, Yufutsu, Kushiro, Kiritappu, Hanasaki, and Nemuro on Hokkaido, the northernmost main island of Japan. Open circles represent survey sites in seaports and airports; closed circles represent survey points of wild settings like forests.

Honshu, Shikoku, Kyushu, and Tsushima Islands, were screened for anti-hantavirus antibodies to HTNV, SEOV, and PUUV. Seropositive animals were detected in Toyama and Shimane (Table 1). Of 471 *A. speciosus*, 5 (1.1%) were seropositive by IFA. Two *R. norvegicus* (5.1%) from Toyama were positive for SEOV. No antibodies to PUUV were detected in any rodent species in the southern region of Japan. The IFA titers of seropositive *A. speciosus* to HTNV (1:32 to 1:128) were 4- to 8-fold higher than the titers to SEOV (1:16 to 1:64).

Table 1. Serological screening for antibodies to hantavirus by IFA among wild rodents in Honshu, Shikoku, Kyushu, and Tsushima Islands (2000–2003)

Rodent species	Seroprevalence at											Positive rate (%) to		
	Aomori	Niigata	Toyama	Yamanashi	Shizuoka	Shimane	Tokushima	Miyazaki	Tsushima	Kagoshima	Osaka	HTNV	SEOV	PUUV
A. speciosus[a]	0/14[b]	0/6	4/223	0/7	0/19	1/69	0/2	0/69	0/33	0/1	0/28	**5/471 (1.1)**	4/471 (0.84)	0/471 (0)
A. argentius	—	—	0/9	0/7	0/9	—	—	0/17	0/7	—	—	0/49 (0)	0/49 (0)	0/49 (0)
M. montebelli	—	—	0/11	—	—	—	—	—	—	—	—	0/11(0)	0/11 (0)	0/11 (0)
E. smithi	—	—	0/11	—	—	—	—	—	—	—	—	0/11 (0)	0/11 (0)	0/11 (0)
M. minutus	—	—	—	—	—	—	—	—	0/1	—	—	0/1 (0)	0/1 (0)	0/1 (0)
M. musculus	—	—	—	—	—	—	—	—	0/4	—	—	0/4 (0)	0/4 (0)	0/4 (0)
R. norvegicus	—	—	2/39	—	—	—	—	—	—	—	—	2/39 (5.1)	**2/39 (5.1)**	0/39 (0)
C. dsinezumi	—	—	—	—	—	—	—	0/1	—	—	—	0/1 (0)	0/1 (0)	0/1 (0)
U. tapoides	—	—	—	—	—	—	—	0/5	—	—	—	0/5 (0)	0/5 (0)	0/5 (0)
Total	0/14	0/6	6/293	0/14	0/28	1/69	0/2	0/92	0/45	0/1	0/28	7/592(1.2)	6/592(1.0)	0/592 (0)

[a] Seropositive rodent species, the places where seropositive rodents were found, and the number of seropositive rodents are shown in bold face.
[b] Number of seropositive rodents/number of captured rodents.

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Table 2. IFA and FRNT for seropositive rodents in Toyama and Shimane (2000–2003)

Place of survey	No. of positives	Species	IFA titer			FRNT[a]	
			HTNV	SEOV	PUUV	HTNV	SEOV
Toyama	4/223[b]	*A. speciosus*	128	16	<16	20	<20
		A. speciosus	64	16	<16	20	<20
		A. speciosus	32	<16	<16	<20	<20
		A. speciosus	128	16	<16	<20	<20
	2/39	*R. norvegicus*	16	32	<16	NT	NT
		R. norvegicus	16	32	<16	NT	NT
Shimane	1/69	*A. speciosus*	64	64	<16	<20	<20
Total							

[a] FRNT titer was expressed as a reciprocal of the highest dilution which showed 80% or more inhibition of the virus focus formation.

[b] Number of seropositive rodents/number of captured rodents.

Table 3. Serological screening for antibodies to hantavirus (HTN, SEO, PUU) by IFA among rodents captured in field surveys in Hokkaido (2000–2003)

Rodent species	Place of survey					Positive rate (%) to HTNV, SEOV, or PUUV
	Nemuro	Kiritappu	Ebetsu	Tobetsu	Yufutsu	
C. rufocanus	1/1[a]	0/8	3/8	1/125	0/11	5/153 (3.3)
C. rutilus	0/20	—	—	—	0/2	0/22 (0)
A. speciosus	—	—	0/1	0/1	0/4	0/6 (0)
A. argentius	—	—	0/1	—	0/9	0/10 (0)
A. peninsulae	—	—	—	—	0/4	0/4 (0)
R. norvegicus	—	—	—	0/4	—	0/4 (0)
S. caecutiens	—	—	—	—	0/3	0/3 (0)
S. gracillimus	—	—	—	—	0/11	0/11 (0)
S. unguiculatus	—	—	—	—	0/1	0/1 (0)
	—	—	—	—	—	
Total	1/21	0/8	3/10	1/130	0/45	5/214 (2.3)

[a] Number of captured rodents is shown in parenthesis.

Seropositive *R. norvegicus* had slightly higher IFA titers to SEOV (1:32) than to HTNV (1:16) (Table 2). Some of the IFA-positive sera from *A. speciosus* neutralized HTNV (1:20) but not SEOV. Virus gene detection was attempted by RT-PCR in seropositive *A. speciosus*, but none of the samples showed the virus-specific PCR product bands (data not shown).

In contrast, of 214 animals captured in wild settings of Hokkaido, only 5 out of the 153 (3.26%) *C. rufocanus* were found to be seropositive in Nemuro, Ebetsu, and Tobetsu (Table 3). No other rodent species captured in wild settings had antibodies to hantaviruses. In rodents captured in urban or semi-urban settings, namely ports and an airport of Hokkaido, 4.5% (2/44) of *C. rufocanus*, 0.62% (2/321) of *R. norvegicus*, and 6.7% (3/45) of *R. rattus* were seropositive (Table 4). *C. rufocanus* captured in both wild settings and at the Chitose Airport had IFA titers to PUUV ranging from 1:32 to 1:128, while the titers to HTNV were negative or lower than those to PUUV. However, none of these serum samples had detectable levels of antibodies to SEOV. In addition, some positive sera from *C. rufocanus* were subjected to WB analysis with hantavirus rNPs to confirm the specific reactivity of the antibodies. All IFA-positive sera showed specific reactions with rNP, while no bands were observed in WB with IFA-negative sera (data not shown). Seropositive *R. norvegicus* and *R. rattus* were found in the port area of Rumoi, Otaru, and Hakodate. The IFA titers to SEOV and HTNV in these samples were almost equivalent, but antibodies to PUUV were below detectable levels (Table 5). In addition, lung tissues from seropositive *R. norvegicus* and *R. rattus* were subjected to RT-PCR to amplify the viral S segment. The partial S segment (256 nt) was amplified from one seropositive *R. rattus* captured at Hakodate that had a high IFA titer (1:512) to SEOV. The nucleotide sequence of the amplicon showed 96% identity with the SEOV prototype strain SR-11 (data not shown).

In order to examine the epidemiology of human hantavirus infection in an at-risk population, we screened sera from the personnel of the Japan Ground Self-defense Force on Hokkaido. Of 207 blood samples examined, one sample was positive (0.48%) for anti-

Table 4. Serological screening for antibodies to hantavirus (HTN, SEO, or PUU) by IFA among rodents captured in ports and the Chitose Airport in Hokkaido (2000–2003)

Rodent species	Place of survey									Positive rate (%) to		
	Chitose[a]	Otaru	Hakodate	Rumoi	Hanasaki	Ishikari	Kushiro	Wakkanai	Tomakomai	HTNV	SEOV	PUUV
C. rufocanus	2/39					0/5				2/44 (4.5)	0/44 (0)	2/44 (4.5)
A. speciosus	0/5									0/5 (0)	0/5 (0)	0/5 (0)
R. norvegicus	0/12	1/115	0/28	1/56	0/4	0/9	0/3	0/35	0/59	2/321 (0.62)	2/321 (0.62)	0/321 (0)
R. rattus		2/44	1/1							3/45 (6.7)	3/45 (6.7)	0/45 (0)
Total	2/56	3/159	1/29	1/56	0/4	0/14	0/3	0/35	0/59	7/415 (1.7)	5/415 (1.2)	2/415 (0.48)

[a] Airport.

Table 5. Serological confirmation of hantavirus infection in seropositive rodents in Hokkaido (2000–2003)

Type of survey point	Place of survey	Animal number	Rodent species	Positives by IFA			WB	Nested-PCR	FRNT to SEOV
				HTNV	SEOV	PUUV			
Wild setting	Nemuro	N126	*C. rufocanus*	64	<16	128	(+)	NT	NT
	Ebetsu, Ishikari	E36	*C. rufocanus*	<16	<16	32	(+)	NT	NT
		E43	*C. rufocanus*	<16	<16	32	(+)	NT	NT
		E55	*C. rufocanus*	<16	<16	64	(+)	NT	NT
	Tobetsu	T37	*C. rufocanus*	<16	<16	128	NT	NT	NT
Airport and seaports	Chitose[a]	C36	*C. rufocanus*	32	<16	64	(+)	NT	NT
		C40	*C. rufocanus*	32	<16	64	(+)	NT	NT
	Rumoi[b]	R-9	*R. norvegicus*	64	64	<16	NT	(−)	<10
	Otaru[b]	13	*R. norvegicus*	64	64	<16	NT	(−)	<10
		15	*R. rattus*	64	64	<16	NT	(−)	<10
		114	*R. rattus*	128	256	<16	NT	(−)	<10
	Hakodate[b]	NH3	*R. rattus*	256	512	<16	NT	(+)	20

[a] Chitose Airport.
[b] Seaport.
NT: not tested.

Table 6. ELISA and WB for anti-hantaviral antibody-positive blood donor found in the Self-defense Force in Hokkaido

No. of blood donors and positives (%)	Place	Donor	ELISA		WB		IFA		
			HTNV	PUUV	HTNV	PUUV	HTNV	SEOV	PUUV
1/207 (0.48%)	Chitose	#195	0.816	0.113	(+)	NT	128	256	<32
		Control	0.023	0.018	(−)	(−)	<32	<32	<32

NT: not tested.

hantavirus antibody by IFA, with titers of 1:256 to SEOV and 1:128 to HTNV. In order to confirm this finding, we carried out ELISA and WB analysis on the IFA-positive sample (Table 6). For the ELISA, various rNPs were used as antigens to determine the type of infecting virus (Fig. 2). The IFA-positive serum reacted most strongly with recombinant SEOV NP (rNP-SEO50) in the ELISA test. The reaction pattern was similar to that of a confirmed SEOV-infected patient serum (Fig. 2).

Discussion

Despite being surrounded by countries endemic for HFRS, Japan has not had a reported case of HFRS for about 20 years. During this period, however, anti-hantavirus antibodies have been detected in *R. norvegicus* captured in various Japanese ports. Our previous epizootiological surveys revealed that 10% of *C. rufocanus* on Hokkaido had anti-hantavirus antibodies and that this species carried PUUV-related viruses (6, 8). How-

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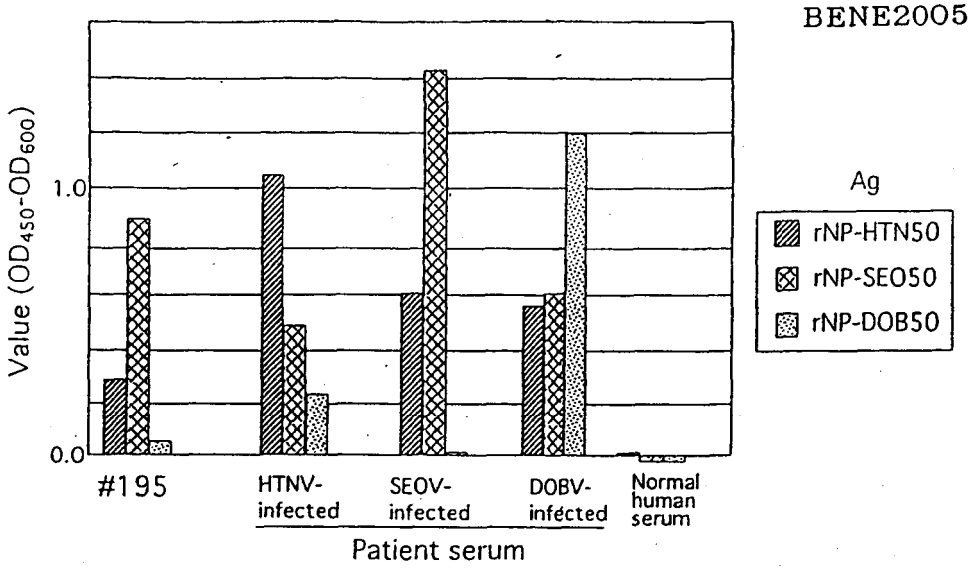

Fig. 2. Anti-hantavirus antibody detection by ELISA. IFA-positive human serum was incubated on a plate coated with different recombinant hantavirus NP with serotype-specific antigenic sites. The plate was incubated with goat anti-human IgG conjugated with alkaline phosphatase. Optical density (OD) was measured at 405 nm.

Table 7. Seroprevalence of hantavirus antibodies in rodents and insectivores of Japan (2000–2003)

Species	Number of seropositive	Number of captured	Positive rate (%)
A. speciosus	5	482	5/482 (1.0)
C. rufocanus	7	197	7/197 (3.6)
R. norvegicus	4	364	4/364 (1.1)
R. rattus	3	45	3/45 (6.7)
A. argenteus	0	59	0/59 (0)
A. peninsulae	0	4	0/4 (0)
C. rutilus	0	22	0/22 (0)
E. smithi	0	11	0/11 (0)
M. minutus	0	1	0/1 (0)
M. montebelli	0	11	0/11 (0)
M. musculus	0	4	0/4 (0)
C. dsinezumi	0	1	0/1 (0)
S. caecutiens	0	3	0/3 (0)
S. gracillium	0	11	0/11 (0)
S. unguiculatus	0	1	0/1 (0)
U. talpoides	0	5	0/5 (0)
Total	19	1,221	19/1,221 (1.6)

ever, epizootiological information on hantavirus infections in indigenous rodents in the southern parts of Japan has been extremely limited. Therefore, this study was carried out to elucidate whether rodent species other than *R. norvegicus* and *C. rufocanus* carry hantaviruses or antibodies to hantaviruses and to examine the prevalence of human infections in a high-risk group, such as personnel of the Japan Self-defense Force.

We found seropositive *A. speciosus* in Toyama and Shimane by IFA screening, as was the case in our previous study (2). In seropositive samples, the IFA titers to HTNV were 4- to 8-fold higher than the titers to SEOV, and the IFA titers to PUUV were all below 1:16. Some of the positive sera from *A. speciosus* neutralized HTNV but not SEOV (Table 2). These findings indicate that *A. speciosus* might carry hantaviruses that are more closely related to HTNV than to SEOV.

We were unable to amplify the virus gene from the seropositive *A. speciosus*. It is possible that the virus was present in seropositive animals at copy numbers too low to permit detectable amplification, or that the primers used in the PCR reaction had insufficient homology with the infecting virus to anneal. Further epizootiological surveys should be conducted to reveal what type of hantavirus is carried by *A. speciosus*. In addition, seropositive *R. rattus* and *R. norvegicus* found

in seaports and at the Chitose Airport could be sources of human SEOV infection, and their presence could pose a threat to people working in these facilities, to travelers, and to quarantine office employees. A higher seroprevalence was reported in workers employed in a reclaimed area where seropositive urban rats were detected (21). Therefore, a larger-scale epidemiological study of hantavirus infection among people associated with the seaports and airports in Japan is warranted.

Further, we found one seropositive individual among the personnel of the Japan Self-defense Force on Hokkaido, as confirmed by IFA, WB, and ELISA. The ELISA result indicated that the person might have been infected with SEOV. Although this group of people has closer contact with *C. rufocanus* than does the general population, no antibodies to PUU-related virus were detected, suggesting that PUU-related virus carried by *C. rufocanus* in this area rarely infects humans.

In this study we detected seropositive animals in *A. speciosus* (5/482, 1.0%), *R. norvegicus* (4/364, 1.1%), *R. rattus* (3/45, 6.7%), and *C. rufocanus* (7/197, 3.6%) among 1,221 animals captured in various areas and settings of Japan (Table 7). These four rodent species may serve as the reservoir animal of hantavirus in the country.

The results of this study, combined with previous findings, suggest that the low occurrence of HFRS in Japan might be attributed to four principal factors: (1) no *A. agrarius* and only a small number of *A. peninsulae*, the main reservoirs of HTNV and AMRV, inhabit Japan; (2) infections caused by SEOV acquired from *R. norvegicus* and *R. rattus* might be mild and easily misdiagnosed; (3) human infection with PUU-related virus from *C. rufocanus* occurs only rarely; (4) the prevalence of hantavirus infection in *A. speciosus* is low.

In this study, we found that *R. rattus* in Japan carries hantaviruses; however, the infecting virus has not been well characterized. Because all hantaviruses must be considered potential human pathogens, we are now analyzing the virus in greater detail.

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